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HUMAN MELANOCYTES EXHIBIT HETEROGENEITY WITH RESPECT TO MELANIN CONTENT AND POSSIBLY CLONOGENICITY. Zalfa Abdel-Malek, Viki Swope, Raymond Boissy, James Nordlund, Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, Ohio.

In many instances cultured human melanocytes exhibit heterogeneity with respect to melanin content. Whether the less melanotic melanocytes represent a less differentiated state than the more melanotic melanocytes or is an independent subpopulation is not known. To investigate these two possibilities we separated neonatal human melanocytes into various subpopulations using a discontinuous Percoll gradient. Cells with least density had the lowest tyrosinase activity, while those with the highest density contained the highest tyrosinase activity and amount of melanin. The same results were obtained when cells were cultured in medium containing TPA or FGF. When the resulting subpopulations were isolated and cultured separately for several passages, they maintained their original phenotype, suggesting that one subpopulation is not a precursor for another. Electron microscopic studies revealed that the least melanotic cells contained premelanosomes and an active tyrosinase enzyme. Thus, these cells are not melanoblasts as previously suggested by other investigators (Bennett DC, et al, J Cell Sci 77:167-183, 1985). Preliminary results showed that melanocytes from different Percoll fractions proliferated at the same rate regardless of their melanin content. Whether the different subpopulations are indeed independent clones or are subject to autocrine inhibitory or stimulatory regulation of their melanization is being investigated.

PREPARATION AND CHARACTERIZATION OF LIPOSOMES FROM STRATUM CORNEUM LIPIDS BY THE EXTRUSION PROCEDURE. William Abraham, and Donald T. Downing, Marshall Dermatology Research Laboratories, Department of Dermatology, Univ of Iowa College of Medicine, Iowa City, Iowa.

Multiple extracellular lipid membranes of the stratum corneum (SC) are believed to constitute the barrier against transepidermal water loss and percutaneous penetration of solutes from the environment.

In the present study, we have prepared and characterized unilamellar liposomes from a lipid mixture containing epidermal ceramides (55% by weight), cholesterol (25%), free fatty acids (15%) and cholesteryl sulfate (5%), approximating the composition of SC lipids. The lipid mixtures were hydrated in an aqueous buffer containing 5 mM tris, 5 mM trimethylamine, 0.5 mM EDTA and 0.02% NaN_3 and extruded through filters of known pore size, at 80°C. After ten passes through the filters, the final pH of the suspension was 8.0. Two different types of filters were tried. The highly porous inorganic membrane (Anodisk) was found to be better suited for dispersing these heterogeneous lipid mixtures than the polycarbonate filter (Nuclepore) used in most extrusion methods. The vesicles were characterized by freeze-fracture electron microscopy and by determining their encapsulation efficiency of C-14 labelled glucose. The vesicles were extremely stable and did not leak any of the encapsulated glucose. Unlike in the sonication procedure, extrusion yielded more homogeneously sized vesicles. These vesicles could be used for permeability studies of various solutes, and would be a relevant model membrane for SC, as they resemble SC in their lipid composition.

ANDROGENETIC ALOPECIA: A REAPPRAISAL OF HISTOMETRIC MEASUREMENT. Abell E, and Kligman A.M. Departments of Dermatology, Univs. of Pittsburgh and Pennsylvania.

Three 6mm punch biopsies were obtained from each of 10scalp reduction specimens. 2 of these biopsies were taken from immediately adjacent sites in the bald vertex area and 1 from the occipital margin. The tissue was processed in the routine way and sectioned by a minor modification of the Headington method to produce serial vertical & transversesections.Measurement of follicular density,mean hair shaft diameter(MSD),and inflammatory infiltrate, were made and counts of the number of follicles in each stage of the hair cycle was made. All biopsies showed reduction in MSD and a shift in the hair cycle toward telogen, the most severe changes being seen in the vertex tissue, but the occipital biopsies were clearly abnormal. Inflammatory infiltration,particularly in the superfical perivascular and perifollicular zones was a consistent finding, and is clearly still present in the occipital areas. Relatively poor correlation of the measured data from the paired vertex biopsies was apparent with the exception of MSD,and this lack of correlation was related to the degree of inflammatory reaction. Despite the poor correlation, the values obtained clearly indicated pronounced changes of the androgenetic type and there was clear separation of these values from those of the less severely affected occipital margin. The inflammatory changes in this condition are not fully understood but are probably of secondary type and related to co-existing seborrheic dermatitis.

METHODS FOR THE DETERMINATION OF CUTANEOUS METABOLISM IN MAN. John Ademola,R.C.Wester, and H.M.Maibach, School of Medicine, University of California, San Francisco,California.

To determine the extent of cutaneous metabolism of test compounds during percutaneous absorption process,Propranolol(26uCi/mmmol,0.013ug/cm^2)was applied to human skin in ethanol vehicle(20ul).Receptor fluid was collected in hourly intervals for total of 24 hrs.Skin was homogenised and radioactivity in the homogenate and receptor fluid was extracted with ethyl acetate under Neutral,pH 7.0; Acidic,pH 2.0; and Basic, pH 9.0 conditions.Substrates and metabolites in the extracts were separated by thin layer chromatography and quatified with a radioactive counter.Of the total percutaneously absorbed Propranolol, 67% was found in the receptor fluid while 9.16% was present in the skin.42% of the absorbed Propranolol was biotransformed,with 30% of the metabolites found mainly in the receptor fluid,while 12% of the metabolites are located in the skin. The radioactive metabolites isolated approximately are as follows 35% Neutral (amphoteric),38% Basic and 27% Acidic compounds.While identities of the isolated metabolites of Propranolol(and of the other compounds) are under investigation, possible metabolites are highlighted.

IMMORTALIZATION OF HUMAN KERATINOCYTES WITH SV40 LARGE T-ANTIGEN: ALTERED KERATIN GENE RESPONSIVENESS TO RETINOIDS . Chapla Agarwal, and Richard L. Eckert, Departments of Dermatology and Physiology and Biophysics, Case Western Reserve University Medical School, Cleveland, Ohio.

Immortalized lines of human keratinocytes are extremely useful for the study of keratinocyte function and may eventually be clinically useful. We have generated an immortal line of human keratinocytes using SV40 large T-antigen. The cells, KER-1, display a nuclear fluorescence when reacted with anti-large T-antigen and are immortal as evidenced by their continued growth in culture (>10 months). KER-1 cells resemble non-immortalized keratinoctyes in that they form confluent monolayers, form cornified envelopes and express normal levels of the envelope precursor, involucrin. The cells also display a normal complement of cytokeratins, although the total keratin content is somewhat reduced.

We have previously reported that keratins are coordinately regulated in human keratinocytes by retinoids (JBC 260:14026, 1985). The KER-1 cells display a similar response to retinoid, with one curious exception. Keratin K7 is not expressed in keratinocytes in vivo or in culture in the presence or absence of retinoids. It is also absent from KER-1 cells in the absence of retinoid. However, addition of trans-retinoic acid or retinol dramatically increased the level of K7. This is a dramatic example of uncoupling in the retinoid regulatory pathway and suggests that the mechanism regulating K7 expression is modified by immortalization so that the K7 gene is rendered retinoid responsive. It is interesting that K7 responsiveness is unmasked, while other keratin genes appear to be normally regulated.

RETINOID SUPPRESSION OF CYTOKERATIN GENE EXPRESSION IS MEDIATED BY TRANSCRIPTIONAL EVENTS. Chapla Agarwal, Ellen A. Rorke, Molly A. Boyce, and Richard L. Eckert, Departments of Dermatology and Physiology and Biophysics, Case Western Reserve University Medical School, Cleveland, Ohio.

We have previously reported that the type II cytokeratins, K5 and K6, are reduced in level when normal keratinocytes are treated with retinoids. This regulation is mediated by a reduction in the level of the corresponding keratin mRNAs (JBC 260:14026, 1985). However, it is not known whether these changes are mediated by transcriptional or post-transcriptional events.

We have examined the mechanism responsible for this suppression using squamous cell carcinoma cells as a model system. Treatment of these cells with low levels (1 to 5 nM) of trans-retinoic acid dramatically suppresses the level of keratins K5 and K6. Only trace quantities of K5 and K6 are observed in treated cultures using antibodies specific for type II keratins. Moreover, RNA transfer blots demonstrate that the mRNAs encoding K5 and K6 are barely detectible in the retinoid treated cultures. The reduction in mRNA level appears to be greater than 100-fold.

Transcription runoff experiments using isolated nuclei indicate a dramatic suppression of transcription of the K5 and K6 genes in the retinoid treated cultures. In addition, RNA stability studies indicate that the mRNAs encoding K5 and K6 are relatively stable. Our results provide the first direct evidence that retinoids regulate cytokeratin gene expression at the transcriptional level.

LIPOXYGENASE-CATALYZED METABOLISM OF THE SKIN CARCINOGEN BENZO(α)PYRENE IN RODENT AND HUMAN EPIDERMIS. Rajesh Agarwal, Haider Raza, and Hasan Mukhtar, Department of Dermatology, Case Western Reserve University and VAMC, Cleveland, Ohio.

The polyaromatic hydrocarbon benzo(α)pyrene (BP) is a ubiquitous environmental pollutant and a known skin carcinogen that is activated by cytochrome P-450 and epoxide hydrolase to metabolites that bind to cellular macromolecules resulting in tumor initiation. In tissues, such as the skin, where this type of catalytic activity is low alternate pathways may be important in the activation of chemical carcinogens and cancer development. Lipoxygenases (LO) are non-heme iron dioxygenases widely distributed in mammalian tissues. In this study the LO-catalyzed metabolism of BP was examined in rodent and human epidermal cytosol. Incubation of ^{3}H-BP (20uM) with arachidonic acid (1mM) and epidermal cytosol (10-125 ug protein) resulted in the metabolism of BP. HPLC analysis showed that 1,6-, 3,6-, and 6,12-quinones are the major metabolites. This metabolism was highest in human (597 pmol BP metabolized/min/mg protein) followed by mouse (80% of human) and rat (30% of human) cytosol; the reaction was dependent on time of incubation, protein and fatty acid concentrations and was inhibited (upto 80%) by nordihydroguaiaretic acid whereas indomethacin had no such effect. Incubation under anaerobic conditions significantly decreased the metabolism indicating that the oxygen iserted is derived from the atomosphere. The metabolites thus formed were shown to bind covalently to epidermal protein. γ-Linoleic acid and 15-hydroperoxyeicosatetraenoic acid but not oleic acid also supported this metabolism, though less efficiently. Our data show that rodent and human epidermis can metabolize BP through arachidonic acid dependent LO pathway.

HMB-45 STAINING OF DYSPLASTIC MELANOCYTIC NEVI DOES NOT CORRELATE WITH MELANOMA RISK. LAhmed, MW Piepkorn, JJ Zone, DE Goldgar, MH Skolnick, LJ Meyer. Depts of Pathology, Medicine, and Medical Informatics, U of Utah and the VA Medical Center, SLC, UT.

Recent immunohistochemical studies of dysplastic melanocytic nevi (DMN) using HMB-45, a melanocyte specific antibody, have proposed that dermal reactivity of histologically atypical lesions correlates with melanoma risk. To evaluate this hypothesis further, we examined nevi diagnosed as dysplastic by NIH consensus criteria from three patient subgroups. These were: (A) 10 subjects with a personal past history of melanoma, (B) 25 subjects with a family history of melanoma, and (C) 15 subjects who were unrelated spouse members of the melanoma risk groups A and B, taken to represent the control population. For each study subject, an unstained section of a compound DMN was stained with HMB-45 by the immunoperoxidase technique. All biopsy material was then examined by two pathologists without knowledge of the patient groups. Ninety percent of the lesions selected showed superficial dermal reactivity to HMB-45 and also demonstrated a positive junctional component. In these samples, HMB-45 reacted with proliferating nevus cells. Nevus cells within the deep dermis that demonstrated histologic features of maturation did not stain positively. Furthermore, our data showed no differences in HMB-45 staining of DMN in the three melanoma risk groups. These data support our viewpoint that dysplasia of many melanocytic nevi represents a phase of melanocytic prolifertaion.

CULTURED EPIDERMAL LANGERHANS CELLS CAN PROCESS AND PRESENT INTACT PROTEIN ANTIGENS. Setsuya Aiba, Stephen I. Katz. Dermatology Branch, National Cancer Institute, Bethesda, MD.

There is considerable controversy regarding the ability of short-term cultured (2-3 days) epidermal Langerhans cells (cLC) to process and present intact protein antigens to specifically sensitized T cells. Some studies have shown that cLC are potent antigen presenting cells (APC) for both haptens and intact protein antigens (J Immunol 39:2551,1987, and PNAS 85:5625, 1988), while in others cLC have been unable to process and present intact protein antigens (JEM 169:1169, 1989). In an attempt to resolve this controversy, we tested the ability of freshly prepared LC (fLC) and cLC from different inbred strains of mice to process and present intact protein antigens to T cell clones and T cell hybridomas in proliferative assays (for clones) and IL-2 assays (for hybridomas). We found that both cLC and fLC from various Ia^{k} mice, including BALB/k mice, process and present intact protein antigens (i.e. hen egg lysozyme (HEL), cytochrome c and ovalbumin) to T cells. These functions are retained in 7-day cLC. In contrast, cLC from Ia^{d} mice do not process or present intact protein antigens, such as HEL and myoglobin, although they can present appropriate peptides to specific T cells and are potent stimulators of allogeneic responses. Furthermore, cLC from F1 (H-2^{k}xH-2^{d}) mice can efficiently process and present intact protein antigens to Ia^{k} restricted T cells, but not to Ia^{d} restricted T cells. Thus, cLC from certain strains of mice are able to process and present intact protein antigens to T cells. Langerhans cells may therefore function as APC not only in skin but also, after migration, in regional lymph nodes.

DECREASED EXCISION REPAIR OF PYRIMIDINE DIMERS INDUCED IN SKIN OF BASAL CELL CARCINOMA PATIENTS BY SIMULATED SOLAR RADIATION. Joseph Alcalay[1,2], Steve E. Freeman[3], Leonard H. Goldberg[1], Margaret L. Kripke[2] and John E. Wolf[1], Department of Dermatology[1], Baylor College of Medicine, Department of Immunology[2], The University of Texas M.D. Anderson Cancer Center, Houston, Texas and the Photocarcinogenesis Program[3], Lovelace Medical Foundation, Albuquerque, New Mexico.

One predominant lesion induced in DNA by UVB is the cyclobutyl pyrimidine dimer formed between adjacent pyrimidines on the same DNA strand. There is evidence that induction of pyrimidine dimers in DNA correlates with tumor incidence. The purpose of this study was to investigate whether people who develop basal cell carcinoma (BCC) have an altered ability to repair UVB-induced pyrimidine dimers in DNA. Twenty-two patients with at least one BCC, aged 31-84 years and 19 healthy volunteers (ages 25-61 years) took part in the study. The patients and the cancer-free subjects were irradiated with 10 graded doses of simulated solar UV radiation with 25% increments on the lower back. 24h later their MED was defined. Then they were given 1MED on 2 different sites on the lower back, and shave biopsies were taken at 0 and 6 hours. The DNA was extracted and the excision repair of UV-induced pyrimidine dimers was determined using a dimer specific endonuclease. At time 0, the average number of dimers was similar in the 2 groups. After 6 hours an average of 22+4% of the dimers were removed in the BCC group compared to 33+4% of those in the cancer-free group (p=0.03). In the BCC group, only 23% of the patients exhibited a repair of more than 30% of the dimers after 6h compared to 53% of the cancer-free subjects. We conclude that patients with BCC have decreased excision repair of pyrimidine dimers induced in their skin by simulated solar UV radiation.

ULTRAVIOLET RADIATION-INDUCED DAMAGE TO HUMAN LANGERHANS CELLS IN VIVO IS NOT REVERSED BY UVA OR VISIBLE LIGHT. Joseph Alcalay[1,2], Leonard H. Goldberg[1], John E. Wolf[1] and Margaret L. Kripke[2]. Department of Dermatology[1], Baylor College of Medicine and Department of Immunology[2], The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Cells in human skin have been reported to contain a photoreactivation repair mechanism that, following exposure to UVA or visible light, repairs UVB-induced pyrimidine dimers. The purpose of this study was to determine whether exposure to photoreactivating light (PRL) would also reverse the UVB-induced morphological alterations in human Langerhans cells. The skin of eight healthy volunteers was exposed to a low dose of UVB radiation (between 0.75 and 1.5 times the minimal erythema dose), and immediately thereafter exposed to PRL from either BLB fluorescent lamps (UVA radiation, 54kJ/m^2) or an incandescent bulb (visible light, 11.4kJ/m^2). These doses of PRL were used because they were effective in the South American opossum and humans. After exposure to UVB radiation, the number of ATPase$^+$ epidermal Langerhans cells was reduced in all subjects to between 21% and 65% of that in unirradiated skin, and the majority of the remaining cells exhibited morphological alterations. Exposure of the UVB-irradiated skin to photoreactivating light did not reverse or reduce these effects. We conclude that UVB-induced morphological alterations of human Langerhans cells are not subject to photoreactivation. These results imply either that pyrimidine dimers are not involved in these effects of UVB irradiation, or that photoreactivation does not occur in human Langerhans cells *in situ*.

THE CUTANEOUS MICROBIOLOGY OF HIV+ PATIENTS: CARRIAGE OF STAPHYLOCOCCUS AUREUS AND CANDIDA ALBICANS. R. Aly, D.J. Bibel, M. Conant, and H.R. Shinefield, Department of Dermatology, UCSF, and Kaiser Foundation Hospital, San Francisco, California.

Microbial ecological studies of the skin in patients with human immunodeficiency virus (HIV) are lacking. The increased incidence of opportunistic infections among this population is a hallmark of AIDS. This study describes a one-year quarterly quantitative and qualitative survey of the microbial flora of 18 male HIV+ patients in various stages of illness and two monthly samplings of 6 HIV- volunteers. Our objective was to determine if HIV infection brings about changes in the normal flora that may pose hazards of subsequent mucocutaneous and skin infections. The microbial floras of the nasal mucosa, oral cavity, and cheek and groin skin were sampled and identified by standard microbiological methods. S. aureus isolates were characterized by antibiotic sensitivity. Data were analyzed according to patient T4 lymphocytes levels. The averge incidence of S. aureus was 49% in the nose and 12% in the groin. The carriage of C. albicans in the oral cavity was 53%. About 83% of patient S. aureus isolates were resistant to penicillin, 80% to oxacillin, 42% to dicloxacillin, and 19% to erythromycin; all isolates were susceptible to cephalothin. Although no unusual patterns in the microbial floras were found, the greatest flux and variety of species occurred in the groin site of subjects with <100 T4 cells/mm^3, and a trend towards smaller total populations with the progress of AIDS was noted. Also, the reciprocal dynamics of normal flora, such as between S. capitis and S. epidermidis and between coryneforms and S. aureus, were confirmed.

COMPARISON OF BASIC CHEMICAL STRUCTURE OF CYSTEAMINYLPHENOL AND IN VITRO ANTI-MELANOMA EFFECT. Frank Alena and Kowichi Jimbow, Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Alberta, Canada.

4-S-cysteaminylphenol (CAP) and its N-acetyl (Ac) derivative (N-Ac-4-S-CAP), are tyrosinase substrates and possess the marked in vivo melanocytotoxicity. To develop a new anti-melanoma agent based upon the melanin synthesis pathway, this report examined in vitro relationship between alterations in the basic chemical structure of 4-S-CAP and their anti-melanoma effects using a microculture tetrazolium assay and SK-MEL-23 human melanoma cell line. We found that tyrosine and its sulfur homologue, cysteinylphenol do not possess any obvious in vitro growth inhibition to melanoma cells. Tyramine, amine derivative of tyrosine and its sulfur homologue (4-S-CAP), on the other hand, showed mild and high cytotoxicities, their IC 50 being 147 μg/ml and 5.0 μg/ml, respectively. Although N-Ac-4-S-CAP was found to be the most effective depigmenting agent in the in vivo studies, 4-S-CAP revealed a higher in vitro growth melanoma inhibition than N-Ac-4-S-CAP (IC 50: 22 μg/ml). These findings may indicate that neither amine group nor the sulfur atom inside the CAP alone is able to enhance the cytotoxicity of 4-S-CAP, although all of them are tyrosinase substrates, but the combination of the two would be the basis for the enhancement of its biological effect. N-Ac group would give a better protective function for biodegradation of 4-S-CAP after systemic administration in vivo, hence, providing a better pharmacologic availability to target cells.

EPITOPE MAPPING OF 230-kD BULLOUS PEMPHIGOID (BP) ANTIGEN FOR HUMAN MONOCLONAL ANTI-BASEMENT MEMBRANE ZONE ANTIBODY 5E. Masayuki Amagai, Takuichi Tsubata, Akira Hasegawa, Keizaburo Miki, Nobuyoshi Shimizu, Takashi Hashimoto, Takeji Nishikawa, Departments of Dermatology and Molecular Biology, Keio University School of Medicine, Tokyo and Tonen Corporate R&D Laboratory, Saitama, Japan.

We previously established a human monoclonal anti-basement membrane zone antibody (MAb-5E), which reacts with the 230-kD BP antigen. We obtained a mouse cDNA clone encoding for about carboxyl-terminal half of the 230-kD protein by immunoscreening a cDNA expression library with MAb-5E. In this study we specified the epitope of the 230-kD BP antigen for MAb-5E by means of deletion mutants of this cDNA clone. Six restricted fragments were subcloned into expression vector (pAT-Trp) and were expressed under control of E.coli tryptophan promotor/operator system. The detection of these antigens were performed by Western blot technique. Three of the six recombinant proteins were recognized by MAb-5E and each of them had a predicted molecular weight. The limited segment which contained the epitope for MAb-5E was located between Bgl II site and Stu I site. This region encoded 144 amino acids and was 296 amino acid upstream from the carboxyl terminal end of the mouse BP antigen. This region was highly conserved between mouse and human and it showed a unique predicted structure of repeated α-helix and β-sheet. The detailed epitope mapping using synthetic peptides is in progress. This approach provides valuable tools to define other immunogenic regions using patients' sera and also to study the function of the 230-kD BP antigen.

THE EFFECT OF ENDOTHELIN ON CELLULAR GROWTH. Alfred F. Alvarez, Matthew H. Katz and Vincent Falanga. Department of Dermatology and Cutaneous Surgery, Miami, Florida.

Endothelin (ET) is a peptide (MW 2,492) produced and secreted by endothelial cells and is the most potent vasoconstricting substance known. It appears to act by increasing cellular calcium uptake. Recently, ET has been found to enhance DNA synthesis by rat vascular smooth muscle cells. ET has been purified to homogeneity and its cDNA cloned. In this study, we investigated the effect of ET on DNA synthesis by human dermal fibroblasts (HDF) and umbilical vein endothelial cells (UVEC). Cells were seeded at 10,000/cm^2 in optimal growth medium, either 10% FBS Dulbecco's Modified Eagle's Medium for HDF or Endothelial Growth Medium-Umbilical Vein for UVEC. The table shows the mean dpm x10^{-3} of ^{3}H-thymidine incorporation ± SD after incubation of cultures with ET for 24 hours.

	Concentrations EG ng/ml			
	0 (control)	0.075	1.25	10
HDF	63 ± 4.8	29 ± 4.5$^+$	39 ± 3.1^{++}	54 ± 4.8
UVEC	24 ± 8.0	37 ± 5.7	27 ± 4.0	29 ± 9.2

+ = $p < 0.001$ ++ = $p < 0.01$

Thus, endothelin in the range of 0.075 ng/ml to 1.25 ng/ml causes a significant decrease in DNA synthesis of human dermal fibroblasts, but not of endothelial cells. The modulation of cellular growth by this novel endothelium-derived peptide may be important after tissue and endothelial cell injury.

MANOALIDE INHIBITS EDEMA AND NEUTROPHIL INFILTRATION IN PHORBOL ESTER TREATED MOUSE EAR. L.D. Amdahl, J.A. Goni and G.W. De Vries, Department of Biological Sciences, Allergan, Inc./Herbert Laboratories, Irvine, CA 92715

Topical application of 12-0-tetradecanoylphorbol-13-acetate (TPA) to mouse skin induces edema, epidermal hyperplasia and leukocyte infiltration. We investigated the effect of the sesterterpenoid manoalide on TPA-induced edema and neutrophil infiltration in mouse ear. In our studies, edema peaked 6-8 hours after TPA treatment (2 ug/ear), while inflammatory cell infiltration peaked at 24 hours. Neutrophil infiltration into ears was determined by measuring myeloperoxidase (MPO) activity in tissue homogenates. MPO enzyme is found primarily in neutrophils. Topical manoalide inhibited TPA-induced edema (IC_{50} = 0.3 mg/ear) and MPO activity (IC_{50} = 0.15 mg/ear) at 6 hours post-TPA treatment. A single application of manoalide also inhibited these responses at 24 hours. Inhibition of cell infiltration may be due to a direct effect of manoalide on neutrophils, since we observed that manoalide (at 0.5 uM) inhibited human neutrophil chemotaxis in vitro, using an under-agarose chemotaxis assay. Other anti-inflammatory agents, such as dexamethasone and indomethacin also inhibited TPA-induced edema and MPO activity. These data demonstrate that manoalide is an effective inhibitor of both edema and neutrophil infiltration, and that the TPA-inflammation model may be useful in evaluating this class of anti-inflammatory drugs.

ENHANCED COLLAGEN GENE EXPRESSION IN FIBROBLAST CULTURES TREATED WITH all-trans-RETINOIC ACID: EVIDENCE FOR UP-REGULATION OF THE α2(I) PROMOTER ACTIVITY. Anne Marie Angeles, Veli-Matti Kähäri, Yue Qiu Chen, Francesco Ramirez and Jouni Uitto. Jefferson Medical College, Philadelphia, PA, and Mt. Sinai School of Med., New York.

Previous studies have suggested that topical application of all-trans-retinoic acid (RA) can alter the metabolism of collagen in the skin. In this study, the effect of RA on type I collagen promoter activity was tested in transient transfections of cultured fibroblasts. Mouse NIH-3T3 cells were grown under serum-free conditions and transfected with a human α2(I) collagen promoter/CAT reporter gene construct. After a 24-h incubation following the transfection, RA was added in concentrations varying from 10^{-5} to 10^{-9} M. The cells were harvested 24 h later, and CAT activity, an index of the α2(I) collagen promoter activity, was determined by incubation with [^{14}C]chloramphenicol as substrate. Incubation of cells with increasing concentrations of RA resulted in enhanced collagen promoter activity; the highest enhancement, ~14-fold, was noted at 10^{-5} M concentration. Parallel transfections with pEP5CAT, an elastin promoter/CAT construct, or with pBSOCAT, a promoterless CAT construct, revealed that the expression of these plasmids was not affected by RA. Assay of mRNA steady-state levels in cells incubated with RA under similar conditions also suggested activation of type I collagen gene expression. Thus, our results suggest that RA, under culture conditions utilized, upregulates collagen promoter activity. These observations may relate to clinical and histopathologic observations made in patients treated with RA for cutaneous aging.

ACITRETIN FISH OIL COMBINED FOR PSORIASIS; CLINICAL RESPONSES AND SERUM LIPID PROFILE. Ashley, J.*, Edelstein, J., Lowe, N.J., Los Angeles and Santa Monica, California.

Acitretin has been shown to be effective for treating moderate to severe forms of psoriasis. It may produce several systemic side effects including hyperlipidemia. Fish oil oral supplements containing omega-3 fatty acids have been shown to have a mild clinical effect in improving psoriasis as well as to inhibit retinoid-induced hypertriglyceridemia. We therefore studied a group of 17 psoriasis patients being treated with 0.5mg/kg Acitretin daily. Clinical grade scores of severity and blood studies were taken monthly. Six patients who showed hypertriglyceridemia (>250 mg/dl) after 1 month on Acitretin, also began receiving 10 capsules (3g of omega-3 fatty acids) daily. After 1 month on this dosage of fish oils, the retinoid-induced hypertriglyceridemia decreased significantly in these patients, from a mean of 362±94 mg/dl to 253±124 mg/dl. There were no significant changes in serum total cholesterol, HDL-cholesterol, Apo AI or B with fish oil supplementation. In addition, patients taking Acitretin plus fish oils cleared faster and more completely than Acitretin patients (65% versus 50%) at 3 months therapy. These results suggest a useful adjunctive effect for fish oils with acitretin treatment.

TGF α, β INDUCED HIGH EXPRESSION OF IL6 mRNA IN KERATINOCYTES Yoshinori Aragane, Hidekazu Yamada and Tadashi Tezuka
Department of Dermatology, Kinki University School of Medicine, Osaka, Japan

Our recent study showed that IL-6, produced by keratinocytes (KC), worked as an autocrine growth factor for themselves. Also, we showed that TGF α, β stimulated IL-6 production in normal and K-TL-1 cell lines. Recently, other investigators reported that TGF α, β are the members of developmentally important growth regulatory polypeptides for KC. In order to know the autocrine and paracrine growth mechanisms of KC, IL-6 mRNA expression was investigated under an addition of TGF α, β. In normal human KC, high levels of IL6 mRNA were present in the induced but not in the uninduced cells as judged by Northern hybridization to a cloned human IL6 cDNA probe. In K-TL-1, which was a trichilemmoma cell line and constitutively expressed IL6mRNA, IL6mRNA levels peaked 24-48 hours after the addition of TGFα, β. However, this increase in IL-6 mRNA level was independent from the concentrations of TGF α, β (1-100 ng/ml). By biological assay using MH60 hybridoma (IL6 dependent cell line), the results obtained from the Northern blot analysis was also confirmed. These results suggest that TGF α, β upregulates the IL-6 production in KC, and that a cytokine network may exist in the epidermis, which regulates inflammation and would healing.

USE OF AFFINITY-PURIFIED ANTIBODIES TO QUANTITATE AND LOCALIZE PARATHYROID HORMONE-RELATED PEPTIDE (PTHRP) IN SKIN. Ercem J. Atillasoy, William J. Burtis and Leonard M. Milstone, VA Medical Center, West Haven, CT and Yale Medical School, New Haven, CT.

Human Keratinocytes in vitro secrete PTHRP whose function is unknown and whose localization in skin in vivo is uncertain. The amount of PTHRP extracted from cultured keratinocytes was 420 pM, while that from human foreskin epidermis was <4pM, as measured in a sensitive PTHRP 1-74 immunoradiometric assay. We have prepared rabbit polyclonal antibodies to synthetic PTHRP's and made region-specific antibodies by affinity purification over columns of synthetic peptides representing the 1-36 and 109-138 regions of the peptide. The peroxidase-anti-peroxidase (PAP) immunohistochemical method was employed in an attempt to localize PTHRP in keratinocyte cultures and skin. While unpurified polyclonal antibodies routinely stain suprabasal keratinocytes as reported by others (J. Bone Mineral Res. 4:273-278), affinity-purified antibodies fail to stain sections of normal skin, squamous cell carcinomas, and cultivated keratinocyte epithelia. The flow-through from affinity purification retains suprabasal staining of keratinocytes. Commercially available monoclonal antibodies to PTHRP similarly fail to stain the aforementioned tissues. Western blots performed on keratinocyte extracts using unfractionated polyclonal antisera show numerous protein bands. The flow-through, but not the affinity-purified PTHRP-specific Ab's, show an idential pattern - an indicator of non-specificity. We therefore conclude that the true localization of PTHRP in skin has not yet been established.

IMMUNOPATHOLOGY OF PRURITIC URTICARIAL PAPULES AND PLAQUES OF PREGNANCY. Iris K. Aronson, Stephanie Vomvouras, Virginia C. Fiedler, Shirley Bond. Dept. of Dermatology, University of Illinois, Chicago, IL.

Pruritic urticarial papules and plaques of pregnancy (PUPPP)/Polymorphic Eruption of Pregnancy (PEP), the most common dermatosis of pregnancy with a broad clinical spectrum is a process whose etiology remains unknown. Between 1979 and 1989 we saw 56 patients with the diagnosis of PUPPP/PEP. Direct immunofluorescence (DIF) biopsies of skin lesions were obtained in 43 of the 56 patients. Immune reactants were present in the DIF biopsies of 11 patients and included IgM, IgA and/or C_3 in dermal blood vessel walls. IgM was present in blood vessels in five patients - IgM alone in two. C_3 deposition in blood vessel walls was present in nine patients, by itself in five, in association with IgM in three and IgA in one. The dermal/epidermal junction (DEJ) showed some granular deposition of IgM in four patients, and fine speckles of C_3 in three, either all along the DEJ or focally in some papillae.

In the 11 patients with immune reactants, the eruption occurred in the third trimester in five, in the second trimester in three, and in the first trimester in three. One patient was an IV drug abuser, one patient had underlying sarcoidosis and the remaining nine patients had no remarkable clinical findings. The eruption subsided in one patient at the time of spontaneous abortion.

The etiology and pathogenetic mechanism of PUPPP/PEP are not understood. Of our 43 PUPPP/PEP patients who had DIF biopsies, 11 (25%) had immune reactants present compatible with the presence of circulating immune complexes (CIC), suggesting an immune etiopathogenesis.

THE INCIDENCE AND RELATIONSHIP OF MELANOCYTE AUTOANTIBODIES IN SMYTH LINE CHICKENS. Lisa M. Austin, Raymond E. Boissy, Bruce S. Jacobson*, and J. Robert Smyth, Jr.**. Dept Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH; and *Molecular and Cellular Biology Program and **Dept Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA.

The development of feather amelanosis in Smyth line (SL) chickens, a model for vitiligo, can be suppressed by neonatal bursectomy (Boissy, et al, Cell Tissue Res 235:663, 1984). This observation suggests the involvement of antibodies in the pathophysiology of SL vitiligo. In this study we characterized melanocyte specific autoantibodies in sera from various SL chickens and correlated their appearance with the development of amelanosis. Initial indirect immunofluorescence studies indicated the presence of melanocyte autoantibodies in the sera of SL which were not present in control Light Brown Leghorns. Western blotting experiments confirmed anti-melanocyte antibodies in SL chickens. Three bands ranging from 65-75 kD were visible. These autoantibodies can be correlated with the clinical onset of feather amelanosis and are probably the result rather than the cause of melanocyte (pigment) cell death. ELISA cell binding assays indicated at least some antigens are on the cell surface. We conclude there are several autoantibodies, some of which may destroy the melanocytes, others may be elicited by cellular destruction.

DETERMINATION OF THE VIABILITY OF HAIRLESS MOUSE STRATUM CORNEUM/EPIDERMIS. Heidi J. Baca[‡], Prema Palicharla[++], Ronald L. Koch[++], Dennis P. West[‡] and Lawrence M. Solomon[‡], Depts. of Pharmaceutics[‡] and Pharmacy Practice[++], Col. of Pharm. and Dept. of Dermatology[‡], Col. of Med., Univ. of Il. at Chicago, Chicago, IL.

Since mouse SCE is being investigated for use as a model tissue for neonatal SCE, the purpose of this study was to determine viability of hairless mouse stratum corneum/epidermis (SCE). One proposed method to assess viability is to measure anaerobic metabolism of glucose to lactic acid. SCE was isolated following incubation of whole skin (WS) in 20mM EDTA-phosphate buffered saline (PBS). Tissues (SCE or WS) were mounted on Franz cells with Dulbecco's modified phosphate buffered saline containing dextrose (1G/L) in the receiving chamber. Samples of the receptor fluid were taken at 1, 4 and 24h and assayed for lactate. At 1h, lactate produced by mouse SCE was 43.8±8.9ug/mL while WS produced 96.0±16.1ug/mL. At 4h, 117.6±33.3ug/mL lactate was produced by SCE and WS produced 312.3±96.4ug/mL. By 24h, SCE produced 301±200ug/mL and WS produced 1,379±462ug/mL lactate. These results indicate: 1) SCE acquired by EDTA-PBS separation yielded viable tissue. 2) the rate of glucose metabolism decreased (from 43.8ug/mL/hr at 1h to 12.5ug/mL/hr at 24 for SCE, and from 96.0ug/mL/hr at 1h to 57.4ug/mL/hr at 24h for WS). 3) the amount of glucose metabolized to lactate was greater with dermis present (WS) in comparison to SCE.

CHROMOSOME 1q LOSS OF HETEROZYGOSITY IN BASAL CELL CARCINOMAS AND LINKAGE ANALYSIS IN NEVOID BASAL CELL CARCINOMA SYNDROME. John W. Bare, Roger V. Lebo, Ervin Epstein, Jr., University of California and S.F. General Hospital, San Francisco, CA.

BCCs occur not only as sporadic events, but also in kindreds (nevoid basal cell carcinoma syndrome - NBCCS) in which susceptibility is inherited as an autosomal dominant trait and multiple tumors develop in affected individuals at a relatively early age. Based on strategies used successfully to identify the tumor suppressor gene whose loss causes susceptibility to retinoblastoma, we have begun a search for such a keratinocyte active gene by testing for loss of heterozygosity in tumors and linkage between chromosomal markers and the NBCCS phenotype.

Because weak linkage of NBCCS to chromosome 1q markers has been reported, we hybridized polymorphic probes from this region to DNA extracted from peripheral blood leukocytes and from sporadic basal cell carcinomas. Of 26 tumors heterozygous for one of two markers at 1q21, 8 (31%) showed partial or complete loss one allele. By comparison, loss of heterozygosity was somehwat less at 1p (19%), distal 1q (13%), and 17p (22%). Preliminarily, the shortest region of overlap of the 1q deletions delimits a small region between the gene for PUM and that for FcγRII, a distance estimated to be 15 cM. However, adding data from one large family we have studied and published data (Farndon and Simmons, HGM 10), linkage to NBCCS was excluded ($Z < -2$) to within approximately 15 cM of PUM. Therefore, it is unlikely that inactivation of a tumor suppressor gene in this region is the primary defect in NBCCS.

A UNIQUE 82KD POLYPEPTIDE IN THE CROSS-LINKED COMPONENTS OF MAMMALIAN EPIDERMIS AND ITS APPENDAGES. Howard P. Baden, Joseph Kubilus, Joseph C. Kvedar, Department of Dermatology, Harvard Medical School, Boston, MA.

Involucrin, keratolinin, loricrin and pancornulins have been identified as precursors of the cornified envelope (CE) of epidermis. We have identified a new unique precursor using a monoclonal antibody (MoAb) prepared against CEs of cultured human keratinocytes. By indirect immunofluorescence (IIF), peripheral staining was observed with the MoAb in the upper layers of human epidermis, cultured keratinocytes and stratified squamous but not simple epithelium. Peripheral staining was observed in the isthmus of the follicle and matrix of nail but staining was cytoplasmic in the internal root sheath (IRS). Similar results were observed with in the dog, monkey, and sheep. Basic polypeptide(s) of 82 kD were identified in Tris-EDTA (TE)and TE with urea and mercaptoethanol (TUM) extracts of cultured human keratinocytes and epidermis which was distinct from plakoglobulin. Proteins of similar character were present in TUM epidermal extracts of several species. Incubation of TE extracts with dansyl cadaverine showed labeling of the band(s). Additional minor immunoreactive bands were present in tissue extracts despite addition of many protease inhibitors which suggests cellular processing of the molecule. Aggregation of the TE soluble form of the protein was noted in freeze-thawed extracts and this was prevented by addition of 0.2 M NaCl to the extraction buffer. Peripheral staining in stratum corneum and reactivity of CE fragments by the MoAb, coupled with the ready aggregation and cytoplasmic location in the IRS of the 82 kD protein(s), suggest its dual role as matrix and CE components.

KERATINOCYTE (KC) PRODUCTION OF A BIOLOGICALLY ACTIVE MONOCYTE CHEMOATTRACTANT. JNWN Barker, ML Jones, C Swenson, RS. Mitra, JT Elder, J.C. Fantone, PA Ward, VM Dixit and BJ Nickoloff. Departments of Dermatology and Pathology, University of Michigan, Ann Arbor.

Monocytes together with T lymphocytes preferentially accumulate in the epidermis and along the dermal-epidermal junction in inflammatory skin diseases. To determine whether (KCs) elaborate a specific monocyte chemotaxin, normal human KC were grown as monolayers in 10 cm^2 petri dishes containing 5 ml serum free, low calcium medium (KGM) with interferon-gamma (IFN-γ, 100 U/ml) or tumor necrosis factor-alpha (TNF-α, 250 U/ml). At 16 hours, the chemotactic activity of the media was assayed via 48-well micro-chemotaxis plates with f-Met-Leu-Phe as the positive control and purified human peripheral blood monocytes as the target cell. A dose response revealed that directed monocyte migration (chemotaxis), up to 60% of maximum f-Met-Leu-Phe reaction, could be elicited with unconditioned KC supernatants. This activity was increased up to nine fold in the presence of IFN-γ, alone or in combination with TNF-α.

Using a rabbit polyclonal antiserum to monocyte chemotaxis and activating factor (MCAF), a protein of Mr 13000 (the predicted size for MCAF) was immunoprecipitated from both the supernatants and cell lysates of metabolically labeled KC. MCAF was detected in the absence of cytokine and increased in the presence of IFN-γ alone or IFN-γ and TFN-α in combination. Subsequently, constitutive expression of MCAF mRNA was detected in cultured KC by RNA blot hybridization using a specific cDNA probe. At 8 hours, IFN-γ produced a 4-fold increase in mRNA while IFN-γ and TNF-α acted synergistically to produce a 30-fold increase in MCAF mRNA. Interleukin-1 and phorbol esters as well as TNF-α alone had no effect, while elimination of epidermal growth factor (EGF) from the medium failed to inhibit transcription. Constitutive expression of MCAF mRNA was also demonstrated in total cellular RNA extracted from keratome specimens of normal human skin (N=8).

These studies demonstrate that KC, both in vitro and in vivo, constitutively elaborate a functionally relevant monocyte chemotaxin, the modulation of which appears dependent on the presence of IFN-γ, a T cell-derived cytokine that is thought to play a critical role in KC/mononuclear cell interaction.

bFGF AND TGF-ALPHA STIMULATE EPIDERMAL WOUND REPAIR IN A NOVEL IN VITRO/ORGAN CULTURE MODEL OF CHRONIC HUMAN WOUNDS. L Barazani, DM Carter, and J Krueger, The Rockefeller University, NY, NY.

Re-epithelialization of skin wounds requires migration of epidermal keratinocytes across a dermal surface with associated keratinocyte proliferation or growth activation. To study the impact of specific growth factors on human epidermal wound repair, we have developed an in vitro organ culture of human skin in which epidermal migration across a dermal equivalent can be studied under controlled conditions. Small fragments of full-thickness human skin are placed on a dermal equivalent composed of human skin fibroblasts embedded in a matrix of bovine type I collagen. Epithelial outgrowth is assessed after incubation for 6-14 days at an air-fluid interface. Effects of fetal calf serum, fibroblast density, and specific growth factors on epithelial outgrowth have been measured in this system.

Control cultures showed average radial outgrowth of 0.8mm (n=10). Addition of recombinant human bFGF or TGF-alpha (10ng/ml or 100ng/ml) increased this outgrowth to 2.5mm and 2.6mm respectively. Reduction of fibroblast concentration in the dermal equivalent by 50% caused significant decrease in bFGF-stimulated outgrowth, whereas TGF-alpha-stimulated outgrowth was not affected. Results suggest that bFGF may exert its effect indirectly via dermal fibroblasts, whereas TGF-alpha may act directly on epidermal keratinocytes. Effects of recombinant bFGF and TGF-alpha were more marked in this epidermal migration assay than in normal human keratinocytes in monolayer culture at the same growth factor concentrations. We conclude that this in vitro system is useful for comparing growth factor efficacy in wound healing.

CHANGES IN THYMIDINE METABOLISM WITH DIFFERENTIATION IN HUMAN KERATINOCYTES IN VITRO. S.K. Barnett, P.M. Schwartz and L.M. Milstone, Department of Dermatology, VA Medical Center, West Haven, CT, and Yale University, New Haven, CT.

Human keratinocytes in vitro salvage extracellular thymidine (TdR) and convert it to nucleotides prior to utilization for DNA synthesis. Keratinocytes also rapidly catabolize TdR to thymine (J. Invest. Dermatol. 90:8, 1988) We therefore compared catabolic and anabolic pathways of 3H-TdR usage by basal-like keratinocytes (grown to 75% confluence in MCDB 153 in 0.1mM Ca++) and by differentiated keratinocytes (Obtained after 24 hrs in suspension culture in DMEM with20% FBS, 1mM Ca++ and 1% methylcellulose). The fate of 5nmol 3H-TdR (2.5uM) was determined after 30 min. exposure to $5x10^6$ cells. Both populations of cells catabolized 40% of TdR to thymine. Basal-like cells formed 0.08nmol of 3H-nucleotides and incorporated 0.03nmol of 3H-TdR into DNA. In contrast, in differentiated cells nucleotide synthesis was <2% and incorporation into DNA was <1% of that in basal cells. Anabolic utilization of 3H-TdR by basal-like cells was readily manipulated; it was decreased by inhibiting TdR transport with dipyridamole and increased by inhibiting TdR breakdown with 6-aminothymine or by inhibiting de novo synthesis with methotrexate. Cells exposed to 5uM MTX synthesized 220% more 3H-nucleotides and incorporated 165% more 3H-TdR into DNA than cells not treated with MTX. These inhibitors had no effect on the ability of differentiated cells to utilize 3H-TdR for nucleotide or DNA synthesis. Our data suggest that anabolic use of TdR is lost with the onset of differentiation in keratinocytes.

DNA REPAIR IN COCKAYNE SYNDROME AND XERODERMA PIGMENTOSUM LYMPHOBLASTOID CELL LINES MEASURED WITH PLASMID IRRADIATED WITH ULTRAVIOLET LIGHT. S. F. Barrett, R. E. Tarone, J. H. Robbins, and K. H. Kraemer, National Cancer Institute, NIH, Bethesda, MD.

We used ultraviolet light (UV)-treated plasmid (pRSVcat) to measure the ability of lymphoblastoid cell lines from 3 Cockayne syndrome (CS) and 2 group A xeroderma pigmentosum (XP) patients to repair UV damage to DNA. The plasmid was treated with 500 J/m^2 of 254-nm UV and introduced into cells by DEAE dextran transfection. Cells were harvested 48 h after transfection and assayed for chloramphenicol acetyltransferase (CAT) activity coded for by pRSVcat. The CAT activity for the CS and XP cell lines was significantly less ($P<0.05$) than that for 3 normal cell lines, indicating defective repair of UV photoproducts. The mean CAT activity in UV-treated plasmid, as a percentage of CAT activity in untreated control plasmid, ranged from 19 to 37% in normal lines, from 7.5 to 18% in CS lines, and from 0.8 to 1% in XP lines. The ability to repair nondimer photoproducts was measured by selectively removing cyclobutane pyrimidine dimers by treating UV-irradiated plasmid with photoreactivating enzyme plus 405-nm light. After photoreactivation the mean CAT activity in normal cells increased slightly but not significantly, while the mean CAT activity in CS lines increased significantly to within the range for the normal cells, and that in the XP cells increased 10-fold but did not increase to normal levels. The results suggest that XP cells are defective in the repair of both dimer and nondimer damage, while the CS cells are defective in the repair of dimers but have apparently normal repair of nondimer photoproducts.

THE EXPRESSION OF ICAM-1 IN INFLAMMATORY SKIN DISEASES. Scott D. Bennion, David A. Norris, Donald B. Mercill, and Lela A. Lee. Dermatology Svc. and Clinical Investigation Dept., Fitzsimmons Army Med. Ctr.; Depts. of Dermatology and Medicine, U. of Colorado School of Medicine and Denver VA, Denver, CO.

It has been proposed that the expression by epidermal keratinocytes of intercellular adhesion molecule-1 (ICAM-1) plays an important role in the interaction of keratinocytes with lymphocytes. ICAM-1 expression in keratinocytes has been shown to be up-regulated by γ-interferon, viral infection, and ultraviolet (UV) light. Lichen planus (LP), erythema multiforme (EM), and subacute cutaneous lupus erythematosus (SCLE) are skin diseases in which keratinocyte interaction with lymphocytes may be important or even prerequisite for disease. In EM, herpes infection frequently precedes skin lesions, and in SCLE, lesions are usually limited to UV-exposed skin. In addition, systemic interferon levels have been reported to be increased in lupus. We wished to determine if ICAM-1 might be involved in the keratinocyte-lymphocyte interactions in these diseases, and if increased ICAM-1 expression in SCLE is a systemic, possibly cytokine mediated, phenomenon.

Biopsies from 6 patients with LP, 6 with EM, 6 with SCLE , and 4 normal subjects were stained with the anti-human ICAM-1 monoclonal antibody RR 1/1. 4/6 SCLE patients had systemic disease. Both lesional and uninvolved skin of SCLE were examined. The normal skin had ICAM-1 staining in basal keratinocytes and dermal vessels. All LP, EM, and SCLE lesions had increased ICAM-1 staining compared to normal skin. LP lesions had increased staining at the dermal-epidermal interface and papillary dermis. The EM biopsies had marked ICAM-1 staining throughout the epidermis up to the stratum corneum and increased staining in the inflammatory infiltrate in the dermis. SCLE lesions had increased staining throughout the epidermis. SCLE uninvolved skin had staining comparable to skin from normal subjects.

These findings lend credence to the theory that keratinocyte expression of ICAM-1 is an important factor in these inflammatory conditions. The increase in ICAM-1 seen in SCLE is a local phenomenon and is probably mediated via local factors such as UV light and inflammatory cells rather than through a systemic effect of cytokines. The diffuse increase in keratinocyte ICAM-1 in EM may be related to herpes virus infection.

CORRELATION BETWEEN THE CHROMATIC COORDINATES OF HEALTHY SKIN AND OF ULTRAVIOLET INDUCED ERYTHEMATOUS SKIN. Bazex J*., Garigue J**., Marguery MC* ., Patau JP**., Roux G**. * Service de Dermatologie La Grave, ** Faculté de Pharmacie de Toulouse, France.

In a previous study we have shown that the variation in the chromatic cutaneous coordinates L* a* b* as a function of the logarithm of the dose of irradiation of the skin are represented by a sigmoïd curve which allowed us to define a threshold dose (DO) beyond which erythema appears. In the present work, we tried to correlate the threshold dose with the chromatic coordinates of healthy skin.

15 healthy caucasian volunteers of either sex and of phototypes I to IV were studied before and 24 hours after irradiation. We used the MINOLTA CR 100 chromameter in the L*, a*, b* system. Data was treated through a computer file.

The graphs representing the results of DO as a function of L*, a*, b* showed interesting correlation. This statistical study was designed to investigate the validity of the two hypotheses in each case : 1) the slope of the correlation line equals zero and/or 2) the correlation coefficient equals zero ; the two tests are not independent. Statistical results allowed us to consider that DO is correlated to a* and to b* with a 1% threshold risk. Note that the correlation between DO and b* is better than that observed between DO and a*. In contrast, no correlation was demonstrated between DO and L*.

In practical terms, this means that the irradiation threshold dose is correlated to the complexion of the skin and can be predicted by colorimetric measurement on healthy skin.

DOUBLE-BLIND EVALUATION OF CAPSAICIN CREAM IN PRURITIC PSORIASIS. Brenda Berberian*, Joel E. Bernstein+, W. Alan Dodd#, Charles N. Ellis**, Jean Rumsfield+, Virginia I. Sulica*, Georgetown Univ. Medical Center, Washington, D.C.*, University of Michigan, Ann Arbor, MI **, Vancover, B.C., Canada #, GenDerm Corporation, Northbrook, IL+.

Substance P (SP), an undecapeptide transmitter of neuronal impulses, has been suggested to be an important mediator of inflammation in a variety of cutaneous disorders, including psoriasis (P). SP levels have been demonstrated to be elevated in P plaques and to dramatically decline with resolution of the P. SP has also been implicated in the pathophysiology of several pruritic disorders. Recent studies have demonstrated that capsaicin, a selective depleter of SP, is effective in relieving itch associated with postherpetic neuralgia and hemodialysis. In the current study, twenty patients with P accompanied by moderate to severe pruritus were treated with either capsaicin 0.025% cream (C) or the cream vehicle (V) under a double-blind paradigm. Both creams were applied four times daily for six weeks. At the end of the study, itching in 73% of C treated patients was either much better or completely gone (36% while itching in 33% of V treated patients was much better or gone (0%). P was cleared (45%) or markedly improved in a total of 82% of C treated patients, as compared to a total of 33% of V treated patients (with only 11% cleared). All of these differences were significant in favor of C treatment. This data suggests that C maybe a useful treatment for the management of pruritic psoriasis.

CELLULAR RETINOIC ACID BINDING PROTEIN (CRABP) LEVELS DURING THE HAIR GROWTH CYCLE. Gail Bazzano, Hala Attia, and Nia Terezakis, Research Department, Touro Infirmary, New Orleans, LA.

Retinoid compounds have been shown to be very important to normal keratinizing epithelial cells. Furthermore, we propose that certain retinoids can increase the rate of hair growth, prolong the anagen phase of the hair cycle and decrease the length of the telogen phase.

In order to study the role of tretinoin in altering the hair growth cycle in an animal model, we used C_3H mice having a first anagen phase lasting approximately 20 days, then a telogen phase lasting 2-5 days. Treatment was begun on day 21 and continued for 60-80 days. CRABP levels were measured in the murine skin in anagen and telogen phases of the hair cycle using the charcoal method and Scatchard plots to determine binding and Kd. The effect of topical tretinoin application on the levels of CRABP was also studied.

The results indicate that CRABP levels and binding are increased in the anagen phase (mean 18.0 pg moles/mg protein) and decrease during the telogen phase (mean 8.0 pg moles/mg protein). Furthermore, tretinoin application, from day 21 on, increased CRABP levels 2 to 3 fold and significantly shortened the telogen phase of the cycle.

These data confirm that tretinoin may play an important role in the hair growth cycle and is capable of altering the dynamics of the normal cycle.

LOSS OF INITIAL CLONE AND EMERGENCE OF A NOVEL GENOTYPE IN CUTANEOUS T CELL LYMPHOMA TREATED WITH PHOTOCHEMOTHERAPY. C. Berger, M. Lee, J. Tien, R. Polvere, P. Heald, P. Benn, E. Bissacia, S. Armus & R. Edelson, Dept. Dermatology, Columbia Univ. New York, N Y, Morristown, N J & Yale Univ. New Haven, CT.

Ten patients with leukemic cutaneous T cell lymphoma (CTCL) were treated with reinfused, autologous leukocytes photoinactivated with psoralen and ultraviolet A light for 6 mo to > 5 yr. All patients presented with clonal T cells detected by a probe for the beta chain of the T cell receptor. Peripheral blood was analyzed to determine if the intial malignant clone persisted.

Extracted DNA, digested with Bam HI, Eco R1, and Hind III, was electrophoresed and transferred to nylon membranes. A ^{32}P-labeled probe for the beta chain of the T cell receptor was used for hybridization.

The results demonstrate loss of the inital malignant clone (9): with no detectable rearrangement (1), a single de novo band representing a low percentage of cells (2), multiple de novo rearrangements (6), or persistence of the initial clone after short-term therapy (1).

Long-term photochemotherapy may alter or delete the initial clonal population in CTCL. Thirty percent of the patients have minimal or absent circulating clonal T cells. While emergence of new clones (60%) may represent natural evolution of the malignancy, the beneficial clinical response and long-term survival of treated patients suggests that a clonal T cell population, possibly targeted to the neoplastic T cell idiotype, may be induced.

HETEROGENEITY OF CYTOKINE PRODUCTION BY HUMAN MALIGNANT MELANOMA CELLS. Cheryl A. Berger, David Damm, Andreas Köck, Thomas A. Luger, & John Ansel, Department of Dermatology, VA Medical Center, Portland, OR and Department of Dermatology II, University of Vienna.

Recent investigations indicate that malignant melanoma cells can produce several distinct cytokines. It is postulated that they may act as autocrine growth factors or may modulate the host immune response to this neoplasm. We have studied individual human melanoma cell lines for the expression and secretion of multiple cytokines; namely interleukin 1 (IL-1), interleukin 6 (IL-6), granulocyte monocyte colony stimulating factor (GM-CSF), and platelet derived growth factor (PDGF). Northern blot analysis using ^{32}P-labeled human cDNA probes was performed on poly A$^+$ selected mRNA isolated from the unstimulated melanoma cell lines and from cell lines stimulated with PMA (20 ng/ml) +/- 1% calf serum for 3 hours. The cell line A375 constitutively expressed the mRNA for IL-1α, IL-1B, and PDGF with enhanced expression after induction with PMA +/- calf serum. There was also induced expression of GM-CSF mRNA by this cell line. In contrast, the G361 cell line expressed only IL-6 mRNA after PMA +/- calf serum induction. The conditioned media from the A375 cells contained IL-1 activity as determined in the D10 bioassay and GM-CSF and PDGF as measured by ELISA. The G361 cell line secreted IL-6 activity as determined by the B9 proliferation assay. The demonstration of heterogeneous expression of cytokines in two human melanoma cell lines suggests that the interactions between these potent immunomodulating and mitogenic factors may have implications in determining the biologic behavior of different malignant melanomas.

STANDARDIZATION OF IMMUNOFLUORESCENT (IF) TESTS FOR IgA-CLASS ENDOMYSIAL (AEmA) AND IgA-class R 1 TYPE RETICULIN (ARA) AUTOANTIBODY MARKERS OF DERMATITIS HERPETIFORMIS (DH) AND CELIAC DISEASE. EH Beutner*, TP Chorzelski**, T Reunala***, V Kumar* and O Hallstrom****, *Dept. of Microbiol. and Dermatol., Univ. at Buffalo, SUNY, Buffalo, NY, USA, **Dept. of Dermatol., Warsaw Academy of Medicine, Warsaw, Poland, and ***Dept. of Dermatol. and ****Dept. of Microbiol., Univ. of Tampere, Tampere, Finland.

AEmA tests on monkey esophagus and ARA tests on rat kidney and liver afford disease specific markers for gluten-sensitive enteropathy (GSE). Almost all active celiac disease and ~3/4 of DH cases are positive for IgA-AEmA in studies in Warsaw and Buffalo. About the same holds true for IgA-ARA tests in Tampere. Double blind studies of 20 coded sera for AEmA and ARA in Warsaw, Tampere and Buffalo with defined IF tests revealed agreements in titers within two dilutions in all three labs with 19/20 AEmA tests and with 17/20 ARA tests. Two of three labs agreed on the remaining titers. The AEmA in 9 of 14 GSE sera (mostly DH) and ARA in 7 of 14 sera were positive. Six controls were negative in all three labs. Interference phenomena, prozones, etc. sometimes cause false negatives.

TUMOR NECROSIS FACTOR-α (TNF-α), INTERFERON-α (IFN-α) AND INTERFERON-γ (IFN-γ) RECEPTORS ON HUMAN NORMAL AND SCLERODERMA DERMAL FIBROBLASTS *IN VITRO*. Brian Berman, M.D., Ph.D. and Juana Wietzerbin, Ph. D. Dermatology Department, Univ. of California, Davis School of Medicine, Davis, CA and V.A. Medical Center, Martinez, CA and I.N.S.E.R.M. Unit 196, Institut Curie, Paris, France.

TNF-α, IFN-α & IFN-γ exert different regulatory effects on proliferation and biosynthetic activities of human dermal fibroblasts (FBs) [Arch Dermatol Res 281:11, 1989; J Invest Dermatol 92:699, 1989]. In as these cytokines bind to specific receptors in order to exert their activities, the expression of TNF-α, IFN-α and IFN-γ receptors on FBs from human adult normal (NL) and scleroderma (SCL) skin cultured *in vitro* were quantitated. Adsorption isotherms were obtained by incubating 2-3 X 10^4 confluent NL and SCL FBs at 4°C for 2 hours with various concentrations of biologically active recombinant human ^{125}I-cytokine. Nonspecific binding, determined in parallel, simultaneous experiments in the presence of at least a 50-fold excess of unlabeled cytokines, was subtracted from total cell-bound counts to calculate specific binding. Replicate experiments revealed 19,742 ± 2,057 high affinity (Kd = 1.15 x 10^{-9} M) TNF-α receptors per NL dermal FB and 15,006 ± 75 high affinity (Kd = 6.75 x 10^{-10} M) TNF-α receptors per SCL FB. No significant difference was detected in the number of TNF-α receptors at cell passages 3, 4 and 5. Cross-linking ^{125}I-TNF-α to its receptor on NL and SCL FBs revealed a 100 kD TNF-receptor complex. NL FBs expressed 3,320 ± 1,781 IFN-α2a and 17,563 ± 1,575 IFN-γ receptors with SCL FBs expressing 3,076 and 16,316 IFN-α2a and IFN-γ receptors, respectively. NL and SCL dermal fibroblasts similarly express IFN-α, IFN-γ and TNF-α receptors.

PREFERENTIAL REPAIR OF 8-MOP PHOTOADDUCTS IN LYMPHOCYTES. P. Bevilacqua, D. Goldminz, R. Edelson, F. Gasparro, Department of Dermatology, Yale University, New Haven, CT.

To determine the distribution and extent of repair of 8-MOP photoadducts in DNA isolated from lymphocytes after treatment with 8-MOP and UVA, we have used three methods. First, the total number of photoadducts was determined by liquid scintillometric analysis of covalently bound [^{3}H]8-MOP. Second, ELISA analysis with a specific monoclonal antibody to the 4',5'-monoadduct (8G1) was used as an independent assay for the quantification of 4',5'-monoadducts. Third, HPLC analysis of enzymatically hydrolyzed DNA was used to determine the distribution of photoadducts. In earlier studies we determined the conditions under which adduct repair occurs in lymphocytes. DNA was isolated from cells on days 0, 2, and 4 after treatment. Liquid scintillation analysis of DNA isolated on day 1 showed that 0.39 adducts per million bases formed. DNA from day 0 and day 2 was analyzed using 8G1. The percent of 4',5'-monoadducts was 33 on day 0 and 53 on day 2 and the percent of crosslinks was 67 and 47, respectively. These results were supported by HPLC analysis of the day 2 sample which was found to contain 46% monoadduct and 54% crosslink. Most of the removed adducts were crosslinks. Therefore, these data indicate that crosslinks are repaired in lymphocytes and 4',5'-monoadducts may escape repair. Thus, HPLC and ELISA methods can be used to determine the specific type of photoadducts formed and repaired in human lymphocytes treated with 8-MOP and UVA.

Non-invasive Fluorometric Determination of Tissue Hematoporphyrin Derivative Levels in Guinea Pig Skin. Eric F. Bernstein, Paul Smith, Gunter Thomas, Walter Friauf, Christopher Black and Angelo Russo, Radiation Oncology Branch, National Inst. of Health, Bethesda, MD

Currently in patients undergoing photodynamic therapy (PDT) with hematoporphyrin derivative (HPD), drug content in skin is estimated prior to light treatment as a function of given dose. This study measured HPD fluorescence in guinea pig skin using pulsed 488 nm light delivered through fiber optics. A hand held device measured tissue re-emitted fluorescence non-invasively utilizing a band-pass filter and a photosensor. A self calibration mode enables normalization of data runs. Readings of relative fluorescence in guinea pig skin were 50, 70, 100, 140, and 200 for HPD doses of 0, 2.5, 5, 10, and 25 mg/kg respectively. These results correlated well with dye extraction from lyophilized tissue extracted with perchloric acid and ethanol, and then measured for fluorescence by a fluorimeter. Tissue extraction readings were 2.4, 5.9, 8.2, 11.9, and 15.5 ng/mg of skin for the given HPD doses of 0, 2.5, 5, 10, and 25 mg/kg respectively. These data demonstrate the ability to measure transcutaneously the amount of HPD present in skin. Photodynamic effect is a function of drug present and light dose administered. Since individuals vary in their metabolism of HPD, accurate quantitation of tumor and skin HPD content will allow for more accurate light dosing thus optimizing PDT.

PURIFICATION AND GENETIC EXPRESSION OF B-NAPHTHOFLAVONE INDUCIBLE NEONATAL RAT EPIDERMAL CYTOCHROME P-450. D.R. Bickers, H. Raza, T.M. Haqqi* and H. Mukhtar, Depts. Dermatology and *Medicine, Case Western Reserve University and VAMC, Cleveland, OH.

Cytochrome P-450s (P-450s) comprise a multigene family whose products are essential for the metabolism of numerous substrates. Several P-450s from liver and other tissues are well characterized whereas epidermal P-450(s) are poorly defined. Topical application of β-naphthoflavone (β-NF, 100 mg/kg) to neonatal rat (4-day-old) skin resulted in a 2.5 fold increase in P-450 content and 4-13 fold increases in monooxygenase activities. One major form of epidermal P-450 was purified and compared with adult rat liver P-450 1A1 purified under identical conditions. The purified epidermal P-450 showed a major band at 54 KDa on SDS-PAGE which comigrated with hepatic P-450 1A1. The specific content of partially purified P-450 was 1.53 nmoles/mg protein, which corresponds to about 42-fold purification. Using Western blotting, the 54 KDa band immunochemically cross-reacted with monoclonal antibody 1-7-1 (P-450 1A1 specific). The purified preparation efficiently catalyzed benzo(a)pyrene hydroxylation when reconstituted with purified NADPH-cytochrome P-450 reductase and phospholipid. These activities were inhibited by α-naphthoflavone and MAb 1-7-1. Peptide fingerprint analysis of the purified epidermal (54 KDa band) and liver P-450 1A1 showed identical 1-7-1 reacting epitopes. Using polymerase chain reaction with oligonucleotides specific for the hepatic P-450 1A1 gene the expression level of P-450 1A1 gene message in skin was substantially increased in animals exposed to β-NF. Our data indicate that epidermal P-450 induced by β-NF is enzymatically and immunochemically similar to liver P-450 1A1.

EXAMINATION OF HUMAN KERATINOCYTES FOR IMMUNOREACTIVE SECRETORY COMPONENT AND FOR SECRETORY COMPONENT mRNA. Carl Bigler, Tamara J. Stevens, J. Clark Huff, Department of Dermatology, Univ. of Colorado School of Medicine, Denver, CO.

Secretory component (SC), the cellular receptor for polymeric immunoglobulins, is expressed by epithelial cells of the gut, respiratory tract, and glands. By immunofluorescence, we have previously detected the expression of SC by cultured human keratinocytes (HK). The purposes of this study were to examine HK by an immunoblotting technique for SC and to detect SC mRNA in HK.

Second passage HK were lysed and analyzed for the presence of SC by an immunoblotting technique with use of a rabbit antibody to SC. HT-29 cells, an adenocarcinoma cell line known to synthesize SC, were used as negative control cells. A band representing SC was detected in lysates of HT-29 cells and HK at about 100 KD. A radiolabelled DNA probe, a 440 BP fragment from the SC gene, was amplified by the polymerase chain reaction (PCR) from the cDNA of HT-29 cells. By Northern blot analysis, mRNA for SC was detected in RNA from HT-29 cells and at a lower level in RNA from HK, but not in RNA from fibroblasts. These results provide further evidence that HK have the capability to synthesize and express SC.

CLONING AND EXPRESSION OF MURINE CD1. Paul A. Bleicher*, Thomas J. Flotte*, Cox P. Terhorst, and Steven P. Balk. Laboratory of Molecular Immunology and *Wellman Laboratory, Harvard Medical School, Boston, MA.

Human CD1 defines a family of cell-surface glycoproteins whose gene and protein structure resemble MHC class I molecules. CD1a, b and c are all present on immature thymocytes, CD1a alone is a marker of Langerhans cells, and CD1c is found on dermal dendritic cells and some B cells. We have recently demonstrated that CD1a and c can be recognized by CD4-/CD8- cytolytic T cells. To further explore the role of CD1 in immune recognition, we have cloned and expressed the murine equivalent of CD1. A lambda zap cDNA library from CBA X B6 thymus was screened at relatively high stringency with a probe for human CD1d. A 1.8 kb cDNA was isolated, sequenced and found to correspond to the partially characterized murine CD1 gene, mCD1.1. The cDNA, by homology with human CD1, encoded a protein with an alpha1,2 and 3 domain; a transmembrane region and short cytoplasmic tail containing a consensus sequence for PKC phosphorylation. Comparative analysis of protein and nucleic acid sequences revealed significant homology with human CD1d but less homology with CD1a, b and c. Northern blot analysis revealed undetectable levels of mCD1.1 mRNA in thymus and spleen of a number of mouse strains.

The cDNA was further subcloned into the expression vector, pSR-neo, and transfected into L cells. Transfected L cells were cloned and screened by Northern blot for mCD1.1 mRNA. Monoclonal Ab were produced in rats immunized with the transfected L cells. Two Ab, 3C11 and 1H1, reacted with mCD1.1 transfected but not mock transfected L cells. These antibodies do not detect mCD1.1 on mouse thymocytes, splenocytes or Langerhans cells. The lack of mCD1.1 expression on these tissues is consistent with the Northern blot results and is in marked contrast with human CD1a, b and c. These results demonstrate that mCD1.1 encodes a potentially functional protein which can be expressed on the surface of transfected cells. However, the distribution of murine CD1 expression in mouse tissues is markedly different from that of human CD1a, b and c. This dichotomy suggests that murine CD1 may differ in function from human CD1.

GAMMA/DELTA T CELLS RECOGNIZE BACTERIAL AND MYCOBACTERIAL ANTIGENS. Nicholas Birchall, Hilary McKenna, and James Watson, Dept. of Immunobiol., Auckland Univ., New Zealand.

The function of thyl γδ DEC's is unknown. Their role in vivo has been postulated to include such diverse functions as cytotoxic activity, removal of stressed autologous cells, response to mycobacterial infection, response to heat shock protein or a similar common antigen, a role in the genesis of autoimmune disease or maybe they are purely vestigial. Using γδ thy 1+ T cells (Northern blot and FACS profile) isolated from the foetal thymus (FT) gland (cultured for 7 days in RPMI and 10% FCS supplemented with rhIL-7) we asked whether these cells would respond to a variety of antigens. Further, the requirement for antigen presentation in the presense of class II cells was assessed using irradiated or paraformaldehyde fixed syngeneic spleen cells. The cells responded to all sonicated irradiated mycobacterial strains tested (Leprae, Bovis, TB, Marinarum and Scrofulosum). Maximal responses were obtained with M. leprae, M. TB and 18KD protein, an immunodominant epitope of M. leprae. The cells also responded to Con A, LPS and an activating CD3 antibody. The response of T cells to LPS is unusual. Bacterial isolates from E. coli strains and pseudomonas also stimulated the cells. Addition of class II cells to all the above antigens inhibited the response. In contrast, the superantigens SEA and SEB and bacterial lysates from strains expected to produce these antigens stimulated the cells only in the presence of class II antigen. Stimulation was measured by cell number and 3Htdr uptake. Time and dose response profiles were obtained for all antigens.

Although γδ T cells respond to a wide variety of stimuli, they have a limited proliferative capacity. This suggests their role may be in early recognition and induction of an inflammatory response prior to the recruitment of effector cells to sites of infection or damage. The response to S. pneumoniae may help explain gutate psoriatic flares after sore throats. Streptococcal epitopes cross-reacting with resident skin T cells have the potential to activate these T cells, thus initiating a cascade of cytokines which may result in the development of psoriasis.

In Vitro Studies of Drug Effects in Psoriasis: Dexamethasone and Acitretin Regulation of Specific Protein Synthesis. E Bloom, B Coulomb, L Dubertret, HI Maibach, H Jones, and JR Polansky. Cellular Pharmacology Laboratory and Department of Dermatology, UCSF, San Francisco, CA, USA, and INSERM Creteil, Paris, France.

Since glucocorticoids (GCs) have provided a mainstay of psoriatic therapy, we evaluated dexamethasone effects in human keratinocytes, fibroblasts, and skin organ culture. Protein synthesis was examined using 2-dimensional electrophoresis of [^{35}S]-methionine-labelled proteins. The "domain of response" of GC-induced proteins was different in human skin fibroblasts compared to in keratinocytes, with major inductions at 35-45 kDa (pI 6-7) for fibroblasts and 50-60 kDa (pI 5-6) for keratinocytes, probably due to separate pathways of gene regulation for GC effects in these cell types. Organ culture studies showed mainly the keratinocyte profile, probably due to a predominance of this cell type and/or its protein synthetic activity. Of potential importance, there were individual groups of GC-regulated proteins observed in psoriatic fibroblasts not seen in matched controls in the 35 to 50 kDa molecular weight range. Identification of these proteins may provide leads to understanding the mechanisms of keratinocyte hyperproliferation in psoriasis. Acitretin appeared to regulate gene products similar to those regulated by GCs. The regulation of psoriatic gene products may be useful for defining relevant drug effects, whether or not the initial pathogenic mechanism involves an immune response or other cellular functions.

IMMUNOMODULATORY PROPERTIES OF CELL WALL MANNAN GLYCOPEPTIDE PREPARED FROM *TRICHOPHYTON RUBRUM*. John S. Blake, Mark V. Dahl, Michael J. Herron and Robert D. Nelson, Dept. of Dermatology, Univ. of Minnesota, Minneapolis, MN.

Trichophyton rubrum causes 90% of all chronic dermatophyte infections. We speculate that mannan glycopeptide components of *T. rubrum* cell wall are immunosupressive. We isolated *T. rubrum* mannan by precipitation with cetyltrimethylammonium bromide. The mannan contained 73% carbohydrate, 18% protein and 2.4% phosphate. *T. rubrum* mannan stimulated proliferation of human mononuclear leukocytes *in vitro* in 2 of 5 donors. In all donors *T. rubrum* mannan inhibited lymphoproliferative responses to multiple stimuli including trichophyton and candida antigens, OKT3 antibody, and a mitogenic lectin from *Phaseolus limensis*. The inhibitory effect of the mannan was attributable to a non-cytotoxic influence on some early step in stimulation of lymphoproliferation which was not stimulus specific. We conclude that mannan from *T. rubrum* inhibits *in vitro* lymphoproliferation in a nonspecific way, and that this effect may be important in the ability of *T. rubrum* to cause chronic dermatophyte infections.

	Cells only	[2]Cells only	*T. rubrum* Ag	*C. albicans* Ag	OKT3 Ab	*P. limensis* mitogen
0	[1]2779	7805	79998	35415	112367	64532
Mannan 125 ug/ml	33951*	6709	53573*	36729	97676*	16416*
250	26683*	3674*	39596*	26334*	97621*	5754*
500	19159*	3767*	21044*	11463*	33703*	1607*

[1]CPM; [2]Unresponsive donors; * $p < 0.05$ as compared to 0 mannan

NUCLEAR RECEPTORS FOR RETINOIC ACID AND THYROID HORMONE REGULATE KERATIN GENE EXPRESSION. M. Blumenberg, I.M. Freedberg, M. Tomic, H. Epstein, and C.-K. Jiang, Department of Dermatology, New York University, New York, NY

Retinoic acid (RA) and thyroid hormone (T3) are essential for epithelial growth and differentiation. Their effects are mediated through nuclear receptor proteins that modulate transcription of specific genes in target cells. To study the effects of RA and T3 on keratin expression, we engineered the promoters of K#3, K#5, K#6, K#10, and K#14 to direct expression of the CAT reporter gene. The resulting constructs were co-transfected with chicken c-erb-A, the T3 receptor gene, and RA-alpha, the RA receptor gene. Activities were measured in the absence and presence of T3 and RA.

The activity of all promoters was reduced by both the T3 receptor + T3 and the RA-alpha receptor + RA, indicating direct suppression of keratin expression by RA and T3. Expression increased several-fold when the T3 receptor was co-transfected without T3, suggesting that the T3 receptor can both suppress and induce keratin gene transcription, depending upon the T3 level.

RA and T3 receptors bind the same palindrome: GGTCATGACC. In the upstream 300 bp segment of the K#14 gene is the sequence GGTGATGAAA which differs in 3 bps from the perfect palindrome. Using site-directed mutagenesis, we replaced the three mismatched bps. The mutant promoter was no longer suppressed by RA and its receptor. T3 and its receptor still suppressed expression. Thus, we have localized a potential site of action of the RA receptor and have shown that T3 and RA effects are separate and independent.

GROWTH AND CYTOLOGIC ABERRATIONS IN MELANOCYTES CULTURED FROM A MURINE MODEL AND PATIENTS WITH VITILIGO. Raymond Boissy, Lisa Austin, Kevin Beato, Estela Medrano, and James Nordlund, Dept of Dermatology, Univ of Cincinnati College of Medicine, Cinti, OH.

An inherent melanocyte defect has been proposed as a possible mechanism for vitiligo. To address this hypothesis, we have cultured melanocytes from the skin of both a murine model for vitiligo (autosomal vit) and patients with vitiligo, and subsequently analyzed the proliferation, morphology, ultrastructure and/or protein profile of the isolated *in vitro* melanocytes. Primary cultures of vit mouse melanocytes grew poorly, could not be subcultured, demonstrated aberrant morphology and died off within two months, dramatically contrasting the behavior of their control C57BL/6J counterpart. Ultrastructurally, these melanocytes demonstrated various stages of autophagocytosis, dilated rough endoplasmic reticulum (RER) and on occasion an unusual dopa reaction product profile. Gel electrophoresis of cell extracts of the pathologic vit melanocytes demonstrated an abundant 66 kD band. Cultured melanocytes from vitiligo patients, expressing both stable and active forms of the disease, proliferated at relatively normal rates, however the ultimate number of passages achieved was less than for control melanocytes. Ultrastructurally, many of these melanocytes demonstrated compartmentalized melanosomes, resembling autophagosomes. In addition, aberrations of the rough endoplasmic reticulum consisting of extensive dilations filled with floccular material and/or concentric ring-like arrangements were observed in some cultures. These results confirm that the melanocyte of the vit mice and some vitiligo patients contain an inherent cellular defect.

INITIAL CHARACTERIZATION OF STRESS ANALYSIS AND KERATINIZATION OF LIFTED VS SUBMERGED DERMAL-EPIDERMAL CULTURED SKIN SUBSTITUTES. ST Boyce, V Hegde, SB Hoath, MM Donnelly, JM Dodick, CP Childress and AP Supp. Depts of Surgery (SB,JD,CC,AS) and Pediatrics (VH,SH,MD), Univ of Cincinnati, and Shriners Burns Institute; Cincinnati, OH.

Cultured skin substitutes (CSS) have demonstrated value for clinical treatment of skin wounds, and for diagnosis of skin toxicity. This study characterizes uniaxial stress analysis and epidermal keratinization of CSS versus time in lifted or submerged culture. CSS were composed of human keratinocytes (HK), fibroblasts (HF) and collagen-glycosaminoglycan substrates, and were cultured in biochemically defined MCDB 153 medium. Duplicate specimens were cultured submerged for 1 week, and then one was lifted to the air-liquid interface during the second week. Samples were collected after 1, 4, 7, 10 and 14 days of culture. Samples (3-5/pt) were prepared for histology and TEM, and were tested for failure load (g) and strain (% change in length) with a computer-driven stepper motor and force displacement transducer. Quantitative data are expressed as mean values:

	(no cells)		CULTURE PERIOD (days)					
					submgd	liftd	submgd	liftd
ENDPOINT	0	1	4	7	10		14	
FAILURE LOAD	16.2	7.1	14.7	10.5	11.2	12.7	6.6	15.2
FAILURE STRAIN	0.24	0.23	0.28	0.26	0.26	0.27	0.23	0.29
CORNIFIED STRATA	n/a	-	+	++	++	+++	++	++++

The data suggest that failure load and keratinization of CSS increase with time of culture if specimens are lifted at culture day 7, and that strain remains relatively constant. Greater strength and keratinization of CSS may improve clinical and diagnostic applications.

COMPLETE FOLLOW-UP AND EVALUATION OF A SKIN CANCER SCREENING IN CONNECTICUT. Jean L. Bolognia[1], Marianne Berwick[2], and Judith A. Fine[3]. [1]Department of Dermatology, [2]Department of Epidemiology and Public Health, and [3]Cancer Control Research Unit for Connecticut at Yale, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

On May 21, 1988, 251 patients were screened for skin cancer in New Haven, Connecticut. A total body skin examination was performed on 98% of the individuals. Based upon follow-up of 93% of patients positive for basal cell carcinoma (BCC), squamous cell carcinoma (SCC), or Bowen's disease, positive predictive values were: 43% for BCC, 14% for SCC, and 50% for Bowen's disease. In the group of patients with atypical nevi, a person with two or more clinically atypical nevi was sixteen times more likely to be histologically confirmed than a person with a single clinically atypical nevus (p=0.003). Eighty patients were screened by both a physician and registered nurse and the crude agreement rate for actinic keratoses was 0.62, for atypical nevi, 0.53, and for BCC, 0.88. Both nurses and physicians overdiagnosed in the screening setting, the nurses more than the physicians. Of the 128 patients advised to seek medical follow-up (positives), 16 did not do so despite several reminders and the most common reasons were lack of insurance, concern only about a possible melanoma, and concern about a benign lesion, not the suspicious lesion.

COMBINATIONS OF INTERFERON ALPHA AND INTERFERON GAMMA DEMONSTRATE A SYNERGISTIC ANTICELLULAR ACTIVITY AGAINST MELANOMA CELLS AND SQUAMOUS CELL CARCINOMA CELLS BUT ACT ADDITIVELY AGAINST MELANOCYTES AND KERATINOCYTES. B.A. Boyd, T.A. Kaspar, S.K. Tyring, M. Brysk, W.R. Fleischmann, Departs. of Microbiology & Dermatology, University of Texas Medical Branch, Galveston, TX.

The antiproliferative effects of interferon (IFN)-alpha A/D and IFN gamma were studied on cultured G-361 human malignant melanoma (HMM) cells and on cultured normal melanocytes. The tetrazolium salt (MTT) assay was employed to determine cell viability after various incubation times with these lymphokines. The anticellular effects of IFNs were evaluated after addition of the individual agents as well as following combinations of these lymphokines. Little difference in antiproliferative activity was observed on the normal melanocytes as compared to the G-361 HMM cells with IFN-alpha A/D. The IFN-gamma showed a somewhat greater effect on the G-361 HMM versus melanocytes at all time periods. The combination of IFN-alpha A/D and IFN-gamma, however, showed a synergistic cytolytic activity on HMM cells but only additive effects against normal melanocytes. Similar findings were observed with normal human keratinocytes and human squamous cell carcinoma cells. Chemotherapeutic agents, other lymphokines, and effector cells are currently under investigation for their antiproliferative activity on these targets. These results indicate that use of IFN-alpha and IFN-gamma in combination may be more effective clinically than high doses of individual IFNs in the treatment of melanoma and squamous cell carcinoma.

ELECTRIC STIMULATION OF HUMAN SKIN FIBROBLASTS - POSSIBLE ROLE OF INSULIN RECEPTORS. **Gerard J. Bourguignon, Wenche Jy, and Lilly Y. Bourguignon, Departments of Dermatology & Cutaneous Surgery, and Cell Biology & Anatomy, Univ. of Miami, School of Medicine, Miami, Florida.**

Although electric stimulation is known to accelerate wound healing, the mechanism by which this occurs has not been determined. Our laboratory has previously reported that High Voltage, Pulsed Galvanic Stimulation (HVPGS) can significantly increase DNA and protein synthesis in growing cultures of human skin fibroblasts (FASEB J. 1: 398-402; 1987). In this study we report that DNA synthesis is not increased by HVPGS if the fibroblast cultures are made quiescent by serum starvation. However, glucose transport is increased following HVPGS treatment of either growing or serum-starved fibroblasts.

Recently, we determined that additional insulin receptors (both high and low affinity) are exposed on the surface of growing fibroblasts during HVPGS treatment (J. Cell Physiol. 140: 379-385 1989). We now report that only high affinity insulin receptors are increased during HVPGS treatment of serum-starved fibroblasts. These results are consistent with the notion that high affinity insulin receptors regulate glucose transport while low affinity receptors are involved in the control of cell proliferation.

DETECTION OF HERPES SIMPLEX VIRUS DNA IN PERIPHERAL BLOOD CELLS DURING ACUTE HERPES LABIALIS USING THE POLYMERASE CHAIN REACTION. SL Brice, SS Stockert, JD Jester, JC Huff, WL Weston, Dept of Dermatology, Univ of Colorado School of Medicine, Denver CO.

Herpes labialis (HL) is generally considered a minor disease, localized to the skin and associated sensory neurons. Little is known about any systemic effects of this common and often recurrent infection with herpes simplex virus (HSV). Hematogenous dissemination of HSV has been shown only in a small number of immunocompromised adults and in neonates, although HSV has been demonstrated in the generalized cutaneous lesions of erythema multiforme and eczema herpeticum. The purpose of this project was to examine the peripheral blood leukocytes of individuals with recurrent HL for the presence of HSV. Blood specimens were obtained from 6 healthy, young adult donors during a typical episode of recurrent HL as well as donors with no history of HSV (HSV seronegative). Leukocytes were isolated by Ficoll separation. The DNA was extracted and examined for HSV using the polymerase chain reaction with HSV specific primers. Quality of DNA was confirmed by amplification with primers for human β globin. HSV was detected in 4/6 blood specimens obtained during acute HL, and in none of the HSV antibody negative samples. We believe this provides evidence for the "systemic" nature of recurrent HL and for a possible mode of viral dissemination in HSV related dermatoses.

VOLTAGE CLAMP ANALYSIS OF ISOLATED STRATUM CORNEUM. Peter Brink, Vincent Walczak and Joel S. Gordon, SUNY Stony Brook N.Y., and Johnson and Johnson Consumer Prods., Inc. Skillman, N.J. The Voltage clamp method has been adapted to study stratum corneum (s.c.) hydration. This technique differs from other electrical analyses of the stratum corneum by employing direct current, which allows the study of both transient and steady state changes in conductance associated with hydration. A voltage clamp analysis of isolated newborn rat s.c.was carried out. Nonhydrated s.c. showed resistances greater than 500 Mohms. A two fold decrease in resistance is observed within an hour of initiating hydration by placing the s.c. on a 5% polyacrylamide gel. Pretreatment of the s.c. with SDS decreases both the hydrated and nonhydrated resistance, as well as altering the kinetics or resistance changes associated with hydration and dehydration as would be expected with a decreased ability to hold water. Altering the viscosity of s.c. with D_2O had no effect on conductance. Concentrations of glycerol calculated to cause a similar increase in viscosity raised s.c. resistance, but also raised the dry weight, suggesting a more direct contribution by glycerol to conductivity changes. Further exploitation of this methodology should allow us to eventually draw the detailed current path through the skin, which will aid in understanding the molecular basis of stratum corneum hydration.

DIFFERENTIAL SENSITIVITY OF DESQUAMIN TO EXOGENOUS ENZYMES. Miriam M. Brysk, Trace Bell, Srinivasan Rajaraman. Departments of Dermatology and Pathology, University of Texas Medical Branch, Galveston, Texas.

We have previously reported on a 40 kD glycoprotein isolated from human stratum corneum. It is an endogenous lectin specific for amino sugars. In aggregation studies with dispersed corneocytes we have shown that squame cohesion was inhibited by amino sugars, by lectins specific for amino sugars, and by the antibody to the glycoprotein. Because of its unique properties we have named the 40 kD lectin "Desquamin". Many structural proteins are degraded in the stratum corneum. A molecule that is an active agent in this environment must survive proteolysis. We therefore examined the sensitivity of desquamin to degradation by a variety of enzymes. Desquamin is resistant to degradation by endoproteinase Glu C, even when boiled in water, SDS, or urea. Under similar conditions it is also insensitive to trypsin, pepsin and α-chymotrypsin. On the other hand, proteinase K completely degraded the molecule. Pronase and papain were active but only at high concentrations. Desquamin was insensitive to α-mannosidase or neuraminidase, even when these enzymes were followed by a secondary incubation with trypsin. Because desquamin is resistant to degradation by many exogenous enzymes, we suspect that this is also true for the related endogenous enzymes that have been shown to be present in the stratum corneum. Its resistance to degradation may be a crucial factor in determing its uninhibited biologic activity, thereby playing a major role in desquamation.

RELATIONSHIP BETWEEN MAST CELLS AND LYMPHOCYTES INFILTRATING BASAL CELL CARCINOMAS (BCC), BA Brod, R Saito, JB Goslen, MD Tharp, JS Deng. Department of Dermatology, University of Pittsburgh, VAMC, Pittsburgh, PA.

Increased numbers of mast cells and lymphocytes have been reported in lesions of BCC; however, the relationship between these two immune cell populations has not been examined. Therefore, frozen sections from 18 nonulcerated BCCs were stained with FITC avidin to identify mast cells (MCs) and monoclonal antibodies specific for B cells and T cell subsets. MCs and T cells were quantified by direct enumeration and expressed as cell no./hpf. MC content in the BCCs ranged from 1.6 to 16 cells/hpf. The majority of mononuclear cells in these tumors were T cells with helper/suppressor ratios ranging from 0.2 to 10.0:1. Two groups of BCCs were evident by these MC and T cell counts. Grp I had > 4 MCs/hpf with 0-10 T cells/hpf while grp II had $\leq$ 4 MC/hpf and > 10 T cell/hpf. No correlation between helper/suppressor ratios and mast cell densities was seen. Thus our results indicate that an inverse relationship between T cells and mast cells exists in BCCs. We hypothesize that this difference in infiltrating immune cell populations may be related to the age and extent of tumor invasion with MCs representing the initial responding cell population and T cells reacting to more advanced tumors.

IN VIVO PERCUTANEOUS ABSORPTION OF CHEMICALS: EFFECT OF MULTIPLE APPLICATION. Daniel A. W. Bucks, Robert S. Hinz, Robert Sarason, Howard I. Maibach, and Richard H. Guy, Departments of Pharmacy and Dermatology, University of California, San Francisco, CA.

The effect of daily topical application on the in vivo percutaneous absorption (PA) of benzoic acid, parathion and salicylic acid in rhesus monkeys was investigated to test further the hypothesis that topical bioavailability, or body burden, of a chemical following chronic exposure may be accurately predicted from the result of a single acute dose experiment. Radiolabeled compounds were applied as an acetone solution to the abdomen at 4 µg/sq cm. No significant change ($p > 0.05$) in PA from that following the initial dose was observed following a 14-day daily-dose regimen for each of the three penetrants considered:

		Multiple application	
Compound	Single dose	1st dose (Day 1)	8th dose (Day 8)
Benzoic acid	66 (19)	85 (19)	89 (19)
Parathion	n.d.	43 (14)	38 (8)
Salicylic acid	59 (32)	67 (17)	78 (18)

[Mean (SD), n = 4; n.d. = not determined]

These results are consistent with recent human in vivo experiments with malathion and corticosteroids.

CIRCADIAN RHYTHMS IN RODENT AND HUMAN EPIDERMIS. William R. Brown, Division of Dermatology, University of Toronto, Ontario, Canada.

Many studies have shown circadian rhythms in cell proliferation in various mammalian tissues but a concensus on the timing of such rhythms has not been established for any tissue. There is a general notion that proliferation may peak at night in rodents but some studies show a different timing, or even two peaks instead of one. There is also a question as to whether circadian rhythms in mitoses peak after the peak in DNA synthesis or at about the same time; examples of both situations are shown in the literature. To help resolve these questions I have compiled and analyzed the data from 94 published circadian rhythm curves of rodent and human epidermis; 24 of rodent S phase, 44 of rodent M phase, 14 of human S phase, and 12 of human M phase. In order to combine data from the different studies, the data from each study were systematically reanalyzed to give the same 6 time points 4 hours apart over 24 hours, calculated as the percent difference from the mean. The plotted results of the combined data showed clear patterns of regular sinusoidal curves for each series. Rodent epidermal S phase peaked at about 4:00 am and M phase peaked at about 8:00 am. Human epidermal S phase peaked at about 4:00 pm and M phase peaked at about 10:00 pm. The peak for S phase in human epidermis occurred about 12 hours after that of rodent epidermis. This 12 hour shift might be expected since rodents are nocturnal and humans diurnal. Mitoses peaked after S phase as expected. The fact that mitoses peaked 6 hours after S phase in humans, compared to 4 hours in rodents, suggests a longer duration for S phase and/or G_2 phase in human epidermis.

DIFFERENTIAL HYDROCORTISONE RETENTION WITHIN THE STRATUM CORNEUM OF THE VULVA COMPARED TO THE ARM. Daniel A. W. Bucks, Howard A. Oriba, and Howard I. Maibach. Department of Dermatology, University of California, San Francisco, California.

The long-term reservoir (substantivity) of hydrocortisone (HC) in the stratum corneum of the vulva and the ventral forearm has been measured in pre- (Pre) and post-menopausal (Post) women ($n \geq 7$). 14-C labeled HC was applied in 20 µl acetone at a chemical dose of 4 µg/sq cm. The dosing site was protected with a ventilated polypropylene chamber which did not impair normal transepidermal water loss. Treated skin was washed 24 hrs post-dosing with soap and water and was re-protected with a new ventilated chamber. One week post-application, the dosing site was again washed, dried, and then cellophane tape stripped 10 times. HC levels, in % applied dose, found in the tape strips were:

	Arm, Pre	Arm, Post	Vulva, Pre	Vulva, Post
Mean (SD)	2.5 (1.3)	1.9 (1.3)	0.74 (0.45)	1.1 (0.3)

HC stratum corneum levels on the arm were significantly ($p < 0.05$, Bonferroni Test) higher than the vulva of pre-menopausal women. In general, HC was uniformly distributed across the stratum corneum and not concentrated in the outer layers. These results show substantial levels of HC present in stratum corneum on day 7 and suggest that age in addition to site affects long-term HC substantivity.

TYRPHOSTINS INHIBIT THE GROWTH OF KERATINOCYTES AND THE EFFECTS OF MINOXIDIL ON MOUSE VIBRISSAE IN CULTURE. A. Buhl[2], R. Gadwood[2], D. Waldon[2], B. Kamdar[2] and V. Groppi[1], Cell Biology Research[1] and Hairgrowth Research[2], The Upjohn Company, Kalamazoo, Michigan

It is postulated that the EGF-receptor signal transduction pathway controls the growth and differentiation of keratinocytes. To test this hypothesis we analyzed the effects of series of low molecular weight molecules, termed tyrphostins, that selectively inhibit signaling through the epidermal growth factor (EGF) receptor by blocking EGF tyrosine kinase activity (Yaish et al Science 242:933 1988). Two of the most potent tyrphostins blocked the growth of EGF-dependent Balb/MK-2 keratinocytes (K_i = 4-5 µM). The morphology of the drug-treated Balb/MK-2 cells was similar to that seen when these cells were made quiescent by removing EGF from normal media. The tyrphostin-dependent growth arrest was reversible with no evidence of toxicity. The growth of HeLa cells and EGF-independent Balb/MK-2 cells was not effected by these drugs. When tyrphostins were added to cultured vibrissae it was found that these agents blocked the effects of minoxidil as measured by metabolic labelling with radioactive cysteine. The tyrphostins by themselves did not effect cysteine incorporation. We therefore conclude that tyrphostins are effective in blocking the effects of EGF in skin keratinocytes and suggest that at least part of the effects of minoxidil in cultured vibrissae require an active EGF-receptor signal transduction.

MECHANISM FOR RECRUITMENT OF MACROPHAGES AND LYMPHOCYTES INTO WOUNDED SKIN. Jian-Ping Cai, Brian Harris, Vincent Falanga, William Eaglstein, Patricia Mertz and Yee-Hon Chin. U. Miami School of Medicine, Miami, FL.

Mononuclear leukocytes infiltrate into wounded skin early after injury and play an important role in the repair process. We have tested the hypothesis that in wounded skin specialized endothelial cells are induced to mediate peripheral blood mononuclear cell (PBMC) emigration from the vasculature into the dermis. Partial thickness wounds made with a keratome on the backs of domestic pigs were excised on days 0 to 9, 12, 15, and 21 days after wounding and tested for the capacity to selectively adhere to PBMC. The results indicated that PBMC overlaid onto sections of wounds from day 4 to 15 adhered selectively to dermal endothelium, with two distinct peaks of adherence observed on day 7 and day 12. In contrast, PBMC did not adhere when overlaid onto frozen sections of normal skin or 0, 1, 2, 3, and 21 day-old wounded skin. Additional studies revealed that monocytes adhered maximally at day 7, whereas T cells adhered optimally at day 12 post wounding. Furthermore, the adhesion process was energy- and magnesium but not calcium dependent and involved surface protein and carbohydrate moieities on PBMC surface. Pretreatment of PBMC with monoclonal antibodies against the LFA-1 adhesive receptors inhibited the binding by >90%, suggesting that LFA-1 adhesive receptors play an important role in the binding process. These studies provide evidence that the recruitment of monocytes and lymphocytes into wounds is an active, dynamic and regulated process mediated at least in part by specific adhesive interactions between mononuclear leukocytes and dermal endothelial cells.

BIOCHEMICAL EVENTS ASSOCIATED WITH HUMAN CUTANEOUS ENDOTHELIAL CELL EXPOSURE TO ENDOTHELIN. Helen A Bull & Pauline M Dowd. Dept. of Dermatology, UCMSM, The Middlesex Hospital, London, UK.

Endothelin-1 (ET-1) is a potent vasoconstrictor which is synthesised by large vessel endothelium and has been implicated in the maintenance of systemic as well as peripheral vascular tone. We have sought to determine whether human cutaneous endothelium is a target for ET-1.

Release of the microvascular endothelial vasodilator prostaglandin E_2 (PGE_2) and intracellular accumulation of c-AMP were measured by RIA in human dermal microvascular endothelial cells (HDMEC) cultured from neonatal foreskin. Responses to ET-1 (10^{-12}-10^{-6}M) were compared to basal responses after 30 min. Histamine was a positive contol. The results are mean ± SEM.

ET-1 inhibited basal release of PGE_2 in a dose dependent manner. Maximum inhibition was with 10^{-9}M ET-1 (58±18%, p<0.05, n=4). Pre-incubation with a G protein activator, pertussis toxin (PT)(0.01-0.1ng/ml) reduced both basal (64±20%, n=2) and ET-1 induced release of PGE_2. Maximum inhibition with 10^{-10}M ET-1 (80±20%, n=2). ET-1 increased intracellular c-AMP in a dose dependent manner, which was further increased by pre-incubation with PT (max. increase 10^{-7}M ET-1, 340±132%, n=2). In both PT treated and untreated HDMEC incubated with 10^{-7}M ET-1, c-AMP accumulation was attenuated by the phosphodiesterase inhibitor IBMX, the Ca^{2+} channel antagonist nifedipine, the intracellular Ca^{2+} chelator TMB-8 and the extracellular Ca^{2+} chelator EGTA. In contrast, ET-1 induced release of PGE_2 was not inhibited by any antagonist and IBMX increased PGE_2 release (316±17%, n=2).

Inhibition of c-AMP accumulation by nifedipine, TMB-8 and EGTA in the absence of potentiation by IBMX, indicates that ET-1 effects are regulated by Ca^{2+} ion flux. The increased c-AMP in the presence of PT but not IBMX suggests that ET-1 activates adenylate cyclase via Ca^{2+} ion flux and does not inhibit phosphodiesterase. Decreased PGE_2 release in PT treated HDMEC is probably secondary to increased c-AMP. These results indicate that ET-1 can inhibit vasodilator release and that the regulation of HDMEC responses to this peptide are complex.

DIOXIN EFFECTS ON GROWTH AND EXPRESSION OF GROWTH FACTOR AND GROWTH FACTOR RECEPTOR IN HUMAN KERATINOCYTES. C. Cain, S. Bundz, R. Coffey, M. Pittelkow, Mayo Medical School, Rochester, MN and Vanderbilt University Medical School, Nashville, TN.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a halogenated aromatic hydrocarbon that alters epidermal proliferation and differentiation and acts as a tumor promoter in skin carcinogenesis. Cellular, biochemical and molecular effects of TCDD on human keratinocytes (HK) are ill-defined. We investigated effects of TCDD on the kinetics of cell proliferation, toxicity, expression of transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) receptor and EGF receptor-ligand binding in HK propagated in serum-free MCDB 153 medium. TCDD (10-100 mM) exerts toxicity in low density, growth factor-depleted cultures of HK. In select conditions, TCDD stimulates HK replication, but has no effect on logarithmic growth of HK under optimum conditions. TCDD enhances the expression of TGF-α, an autocrine growth factor of HK. TGF-α mRNA accumulation is enhanced within 12 h of treatment and persists at 48 h of TCDD exposure. The kinetics and intensity of expression of TGF-α differ from phorbol ester. EGF receptor-ligand binding is inhibited by TCDD. Cellular effects of TCDD on HK growth and toxicity depend on cell density, growth factors in the culture medium and growth state. Modulation of TGF-α expression and EGF receptor-ligand binding are coupled responses mediated by TCDD. Altered growth factor and growth factor receptor expression/activity may induce abnormal epidermal growth/differentiation and tumor promotion *in vivo*.

DIGITAL VASCULAR RESPONSES TO NEUROPEPTIDES AND HISTAMINE AT LOW TEMPERATURES IN RAYNAUD'S PHENOMENON C. B. Bunker, J. C. Foreman and Pauline M. Dowd. Dept. of Dermatology, U.C.M.S.M, The Middlesex Hospital, London. U.K.

It has recently been suggested[1] that in primary Raynaud's phenomenon (RP) there is a selective suprasensitivity to intravenous calcitonin gene-related peptide (CGRP). This is at variance with the demonstration of no difference in the sensitivity of the digital cutaneous vasculature to intradermal CGRP[2]. The responses of the digital vasculature to CGRP and other vasoactive agents at distal digital sites at low temperatures have now been investigated.

8 women with RP and 10 age matched normal women were investigated. At 21ºC, baseline cutaneous blood flow was measured over the dorsum of the distal phalanges of one hand using laser Doppler flowmetry (LDF). An intradermal injection of 25 µl of either 10 µg/ml histamine, 10 µg/ml compound 48/80 (C48/80), 1 µM CGRP, 1 µM substance P (SP) or saline vehicle control was randomly made at each site. After 2 min., blood flow was again recorded and % change over baseline computed. Diameter of the visible flare was measured by planimetry. The subject was then introduced into a temperature and humidity (40%) controlled Environmental Chamber set at 50C and the experiment repeated on the fingers of the contralateral hand.

Results (mean % change over baseline ± standard deviation) are tabulated below.

		SALINE	HISTAMINE	C48/80	SP	CGRP
Normals	21ºC	427±429	755±431	604±275	666±351	407±178
Normals	5ºC	269±158	765±384	641±656	493±224	405±187
RP	21ºC	423±410	756±704	547±332	426±229	360±143
RP	5ºC	158±183	617±385	411±375	456±301	191±72

Kruskal-Wallis one way analysis of variance showed no significant differences between the LDF responses of RP and normals for any of the agonists tested except CGRP. Wilcoxon signed rank tests showed that at 5ºC there is a significant diminution in the response of both RP and normals to saline and in the response of RP to CGRP. These data do not support a suprasensitivity to CGRP in RP but rather indicate a decreased sensitivity in the cold.

1. Shawket S. Dickerson C. Hazleman B. and Brown M. J. *Lancet* 1989; 2: 1354-1356.
2. Bunker C. B. Foreman J. C. and Dowd P. M. *J Invest Dermatol* 1989; 92: 409.

SOMATOSTATIN ANALOGUE (OCTREOTIDE ACETATE) VS. PLACEBO IN THE TREATMENT OF PSORIASIS. A MULTICENTER STUDY. C. Camisa, F. Bagatell, C. Bainbridge, R. Binder, H.I. Katz, J. Kreindler, N. Lowe, A. Martin, Cleveland Clinic Foundation, Cleveland, Ohio.

A double-blind placebo-controlled study of safety and efficacy of octreotide in 150 patients with chronic plaque-type psoriasis was performed. Patients were randomized to receive octreotide 200 mcg, 100 mcg, or matching placebo by s.c. injection b.i.d. for 12 weeks. Efficacy was evaluated by estimating involved body surface area, a 5-point grading scale of overall improvement completed by investigator and patient, and a 4-point grading of erythema, scaling, and thickness of 3 target plaques. Gallbladder ultrasounds were carried out at baseline and at 12 weeks. 124 patients completed the study per protocol. The investigators' and patients' global assessment of disease was improved for both doseage groups of ocetreotide compared to placebo ($p<.05$). Evaluation of thickness and erythema ($p<.05$), but not scaling, were decreased compared to placebo. Beneficial effects were seen within 4-8 weeks of initiating treatment. However, there were no statistically significant changes in involved surface area in any group. Gastrointestinal side effects occurred in 90% of patients treated with octreotide, but only 4/99 dropped out for this reason. 10/99 (10%) patients treated with octreotide developed gallstones compared to 1/51 (2%) placebo-treated patients. Gallstones appeared and disappeared in direct relationship to the administration of octreotide. Without clearing psoriasis, octreotide is definitely superior to placebo as monotherapy.

LIFETIME TOPICAL TREATMENT OF HAIRLESS MICE WITH TRETINOIN. Robert J. Capetola, James A. Mezick and Lorraine H. Kligman, R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey and Department of Dermatology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

The discovery that topical tretinoin reverses some of the effects of photodamage may lead to its chronic application. It was therefore of interest to assess its long-term effects in hairless mice. We treated the dorsal trunk of three groups of female albino mice (age 6-8 weeks) with 1) tretinoin (0.025%), 2) cream vehicle, 3) sham treatment. Applications were three times a week and continued for up to 2 years until mice were sacrificed or had died. Bi-weekly examinations showed no signs of retinoid toxicity, with growth and longevity similar in all groups. One tumor developed in the vehicle group only. Tretinoin-treated skin was smooth and pink, resembling that of younger mice while controls had yellowed, irregularly thickened skin. Histologically, tretinoin-treated skin had a hyperplastic epidermis (~ 8 cell layers) consisting of plump, cytologically normal cells with an abundant cytoplasm. Control skin had the usual 2-4 compressed cell layers. Unlike in controls, blood vessels were readily seen in the tretinoin group. Foci of new normally staining collagen were present in the subepidermal dermis of tretinoin-treated skin. Fibroblasts were large and abundant in these areas. These foci were absent in controls. In addition, mice treated with tretinoin appeared to have an increased amount of elastic fibers. These results suggest beneficial effects in chronologically aged as well as photoaged skin.

THE EFFECT OF VARIOUS RETINOIDS ON HAIR CYCLE DYNAMICS IN THE C_3H MOUSE MODEL. Leonardo Celleno, Maria Tamburro, Ferdinando Serri, Gail Bazzano, and Nia Terezakis. Department of Dermatology, Universita Cattolica del Sacro Cuore, Rome, Italy and Touro Infirmary Research, New Orleans, LA.

All trans retinoic acid (RA) has been shown to alter the hair cycle dynamics in C_3H mice and to shorten the telogen phase of the hair cycle.

In order to determine whether other retinoid compounds which may or may not bind to cellular retinoic acid binding protein (CRABP) can also alter the hair cycle, we applied different retinoid compounds topically to the backs of the C_3H mice from day 21 of the cycle onward to day 90. We also followed the animals histologically and performed determinations of CRABP levels at intervals during the hair growth cycle.

The results of the study showed that the retinoid compounds which were effective in altering the levels of CRABP in the skin of the mice were also capable of altering the hair cycle dynamics and shortened the telogen phase of the hair cycle in the C_3H mice. Compounds were ranked according to their ability (1) to shorten the telogen phase, (2) to bind to CRABP, (3) to alter the levels of CRABP in the skin, and (4) in comparison to minoxidil.

This C_3H mouse model of hair growth may have potential for selecting out retinoids which will be most effective in promoting hair growth and in reversing some of the changes which occur in hair cycle dynamics during the course of androgenetic alopecia in humans.

COMPARISON OF BINDING PROPERTIES OF RETINOIC ACID AND SYNTHETIC ANALOGUES FOR CRABP AND THE NUCLEAR RETINOIC ACID RECEPTORS RARα AND RARβ. M.T.CAVEY, B.MARTIN AND B.SHROOT. Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, Valbonne, France.

The recent discovery of at least three distinct nuclear receptors for retinoic acid (RAR's) has provided a new means of investigating the mode of action of this molecule. At the same time, however, this has cast doubt on the previously presumed role of the cellular retinoic acid binding protein CRABP. The description of the spatio-temporal distribution of the RAR's in cells and tissues, has given some clues as to their function, but our alternative approach to this question has been to seek to identify receptor specific compounds. We have determined the Ka values of synthetic analogues of retinoic acid for rat testis CRABP (by gel permeation), and for RARα and RARβ present in nuclear extracts of cos7 cells transfected with the expression vectors RAROα and RAROβ* (by HPSEC). Using this approach, we confirmed that retinoic acid exhibits a high affinity for CRABP, RARα and RARβ. We have furthermore synthesized retinoic acid derivatives which show no significant binding to CRABP, but which display specificity for either RARα or RARβ, whilst maintaining a high affinity for their respective receptor.

* kind gifts of Drs.P. Chambon and M. Petkovich- Strasbourg, France.

INTERNAL BINDING SITES FOR MSH IN CLOUDMAN S91 MELANOMA CELLS CO-PURIFY WITH COATED VESICLES AND ARE REGULATED BY UVB RADIATION. Ashok K. Chakraborty and John M. Pawelek. Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Internal binding sites for MSH have been observed in Cloudman S91 mouse melanoma cells. Recent analyses of these sites in variant clones which differed from the wild-type in their responses to MSH indicated that expression of the sites is an important criterion for cellular responsiveness to MSH. Here we report further analyses of these binding sites. Using the bivalent cross-linking agent disuccinylimidyl suberate and ^{125}I-β-MSH as a probe, we showed that the internal binding sites, isolated by sucrose gradient centrifugation have an apparent molecular weight on SDS/PAGE gels of 50-53 kDa and are identical, by this criterion, to MSH binding sites on the plasma membrane. Further isolation of the internal sites by differential centrifugation revealed that they co-purify with coated vesicles from the Cloudman cells. A single exposure of the melanoma cells in culture to relatively low doses of UVB radiation (5-20 mJ/cm^2) caused a decrease in internal MSH binding sites and a concomitant increase in plasma membrane sites, suggesting that UVB may promote externalization of the internal sites. Whether this latter possibility is correct remains to be proven, but the notion is consistent with recent evidence that UVB-induced melanogenesis may be mediated through the MSH-receptor system.

A MICROBIOLOGICAL SURVEY OF PATIENTS WITH SEVERE FOOT ODOR. A.L. Cazzaniga, P. M. Mertz, Univ of Miami Sch of Med, Dept Derm and Cutaneous Surg., Miami, Fl

Severe foot odor can be a major problem for both patients and their families. Using an olfactory panel, we screened 14 volunteers and divided them into 4 groups based on clinical signs and symptoms: a) 3 volunteers suffering strong foot odor and Pitted Keratolysis, b) 5 with strong odor and Tinea pedis complex, c) 3 with only strong odor, and d) 3 with no disease and no odor. Quantitative and qualitative bacterial cultures were taken from target interspace. Dermatophyte cultures and potassium hydroxide mounts were made. Results are as follows:

Groups	Total cfu/ml	Organism types
a)	6.6±0.4	*Staphylococcus aureus, S. epidermidis, S. haemolyticus, Pseudomonas aeruginosa, Brevibacterium sp.*
b)	6.6±0.6	*S. saprophyticus, S. haemolyticus, S. aureus, S. epidermidis, S. xylosus, Proteus mirabilis, Providencia alcalificiens, Micrococcus sp., P. aeruginosa*
c)	6.4±0.3	*S. aureus, S. haemolyticus, S. saprophyticus, S. epidermidis, S. simulans,* and *Micrococcus* sp.
d)	6.1±0.5	*S. aureus, S. saprophyticus, S. capitis, S. xylosus, S. haemolyticus.*

We have been unable to determine a relationship between the number or type of bacteria isolated and strong foot odor.

Rat Connective Tissue Mast Cells (CTMC) Express mRNA For A Tumor Necrosis Factor α (TNFα)-like Peptide. **LJ Chan, MD Tharp. Department of Dermatology, University of Pittsburgh, Pittsburgh, PA.**

Studies from our laboratory have shown that freshly-isolated rat CTMC kill specific tumors in vitro. Functional experiments indicate that this CTMC-mediated cytotoxicity results, in part, from the release of a tumor necrosis factor-like (TNF-like) molecule. While immunostaining with polyclonal anti-TNF antibodies confirms the presence of a TNF-like substance in these CTMC, the origin of this cytokine remains undefined. Therefore, the purpose of this study was to determine whether CTMC constitutively generate a TNF-like peptide or acquire this cytokine by phagocytosis from another cell source. Rat CTMC were purified to > 99% homogeneity and incubated for 90 minutes in buffer alone or buffer containing mouse monoclonal IgE anti-DNP antibodies. The cells were washed (X2) and challenged with the antigen DNP-BSA. After 45 minutes, mRNA was extracted from the CTMC populations and hybridized with a recombinant human TNF-α probe. Under moderate stringency hybridization conditions, mRNA for the TNF-like molecule was detected in the sensitized and antigen-challenged CTMC population whereas no detectable mRNA for TNF was evident in unstimulated CTMC. TNF mRNA also was detected in rat macrophages stimulated with LPS while the CTMC-sensitive tumor, WEHI-164, served as a negative control. The results of this study demonstrate that freshly-isolated, rat CTMC are capable of expressing mRNA for the TNF-like peptide, and that the production of this TNF-like substance is closely linked to mechanisms which activate CTMC.

THE SPECTRUM OF CICATRICIAL PEMPHIGOID. Lawrence S. Chan, Craig H. Hammerberg, H. Kaz Soong, Graciela Cantu, Kent Johnson, Kevin D. Cooper, Departments of Dermatology, Ophthalmology, and Pathology, Univ. of Michigan Med. School, Ann Arbor, MI.

Subsets of patients with Cicatricial Pemphigoid (CP) exhibit various combinations of ocular, oral, or cutaneous lesions. In an attempt to immunologically distinguish CP subsets from each other and from Bullous Pemphigoid (BP), we analyzed 51 consecutive patients with CP documented by in-situ-bound linear basement membrane zone (BMZ) immune deposits, as well as 37 patients with BP. 25 % of our CP patients had oral lesions without ocular lesions, 22 % had both oral and ocular lesions, and 53 % had ocular lesions without oral lesions. 90 % of CP patients with skin lesions occurred in association with oral lesions. Linear BMZ fibrin deposits detected by direct immunofluorescence microscopy were present in 49 % of all CP patients, as compared to 8% of BP patients. Linear fibrin as the only immune deposition occurred in 33 % of CP patients with ocular lesions without oral lesions, and in 0 % of patients with oral CP or BP. Indirect immunofluorescence microscopy (IIF) detected anti-BMZ antibodies in only 11 % of 28 CP patients' sera tested on rat tongue epithelium. However, with 1 M NaCl-split normal human skin as substrate, IIF detected anti-BMZ antibodies in 36 % of 14 CP patients' sera tested, all binding to the epidermal side of the separation and all occurring in patients with oral lesions. The sera of these 14 CP patients and 5 BP patients were subjected to Western blot analysis to determine whether CP serum antibodies in our patient group detected epidermal antigens extracted from cultured human keratinocytes. Ten of the 14 were positive, but antibodies occurred in all groups of CP patients. Only one CP serum identified the 230-kD BP antigen. The remaining sera did not bind to a common antigen, reacting variously to bands of 270-kD (3 sera), 205-kD (2), 160-180-kD (2), 130-140-kD (2), 75-100-kD (4), and 40-55-kD (4). In conclusion, CP patients are distinct from BP patients in their clinical findings, in their frequent fibrin deposition along the BMZ, and in their lack of serum antibodies binding the BP antigen. The molecular heterogeneity of epidermal antigens identified by the sera of each CP subset suggest that CP may not necessarily be an antibody-initiated disease.

EFFECT OF ALL-TRANS RETINOIC ACID ON DERMAL COLLAGEN SYNTHESIS IN UVB-IRRADIATED AND NON-IRRADIATED HAIRLESS MICE. S. Chen, I. Kiss and K. M. Tramposch. Dermatology Research, Bristol-Myers Squibb, Co., Pharmaceutical R&D Division, Buffalo, NY 14213.

The effect of all-trans retinoic acid (t-RA) on de novo collagen synthesis in UVB-irradiated and non-irradiated female albino hairless mice (Skh:HR1) was investigated. Animals in the irradiated group were exposed to a cumulative dose of 1.8 J/cm^2 of UVB over a period of 10 weeks. t-RA was then applied topically (0.1% in ethanol, 50 ul daily, 5 times a week) on both irradiated and age-matched non-irradiated animals after the termination of UVB exposure for an additional 10 weeks. Throughout the 20-week period, on a bi-weekly basis, animals were sacrificed and full-thickness skin biopsies were labeled with tritiated proline for the determination of % collagen synthesis. % collagen synthesis was defined as the amount of incorporated radioactivity released by bacterial collagenase normalized by the corrected total incorporation into skin proteins. t-RA had a marked, but delayed effect on collagen synthesis in animals previously exposed to UVB. The effect was not detectable until 6 weeks into the treatment (165% stimulation relative to the vehicle control) and reached a 245% stimulation after 10 weeks of treatment. Interestingly, t-RA had no effect on animals not previously exposed to UVB. These results demonstrate that t-RA stimulates collagen specifically in the UVB-irradiated animals and that % collagen synthesis is an informative biochemical marker for investigation of retinoid-induced repair of photodamaged skin.

CONFORMATIONAL AND PEPTIDE-DIRECTED ANTIBODY IDENTIFICATION OF PHOSPHODIESTERASE IN ATOPIC DERMATITIS. Sai Chan, Jeffrey Kosokoff, Joseph Beavo, and Jon Hanifin, Depts. of Dermatology, Ore. Health Sci. U., Portland, and Pharmacology, U. of Washington, Seattle.

We have previously related immune dysfunction in atopic dermatitis (AD) to deficient cAMP resulting from elevated cAMP-phosphodiesterase (PDE) activity caused by an abnormal, low Km isozyme in AD monocytes. Using three antibodies (Ab's) directed against different regions of the PDE molecule, we now show that PDE may be genetically and functionally distinct in AD.

Cytosolic PDE from isolated blood monocytes was identified by solid-phase immunoadsorption and by SDS-PAGE immunoblotting with: 1) a monoclonal antibody (mAb) against calmodulin (CM) bound to PDE (ACC); 2) a mAb against the CM binding domain of the PDE (ACAP); and 3) a polyclonal Ab against a 17-amino acid C-terminal sequence of the PDE molecule (C-Diff).

Immunoblotting of monocyte PDE's, showed ACC and ACAP binding to AD but not to normal PDE. The abnormal form of AD monocyte PDE was significantly immunoadsorbed by both ACC (72±12%, N=6) and ACAP (56±13%, N=6). Normal cytosols did not differ from non-specific IgG controls. It appears that CM remains bound to the AD monocyte PDE molecule, even with SDS denaturing conditions, allowing ACC recognition. Immunoblotting with C-Diff was positive against both normal and AD monocyte PDE's, indicating a conserved C-terminal peptide, but quantitatively higher in the latter.

These findings suggest that the stably activated, CM-bound PDE is quantitatively increased in AD monocytes, reducing cAMP control of inflammatory hyper-reactivity in atopic dermatitis and other atopic conditions.

IDENTIFICATION OF GROWTH FACTOR ACTIVITIES OF WOUND FLUID COLLECTED UNDER HYDROCOLLOID DRESSINGS. W. Y. John Chen, and Michael J. Lydon, ConvaTec Wound Healing Research Institute, Deeside, Clwyd, United Kingdom

We recently found that wound exudate collected from porcine full thickness wounds dressed with hydrocolloid dressings (HCD) (DuoDERM™) stimulates growth of human dermal fibroblasts in culture. Wound fluid samples were collected from porcine experimental wounds dressed with HCD 24 and 48 hours after initial wounding. The wound fluid samples were dispersed in culture medium, centrifuged to remove insoluble material and sterilized by filtration (0.22 μm membrane). Culture medium (Dulbecco's MEM, 2% foetal bovine serum) supplemented with 5% (v/v) wound fluid resulted in 53.5 ± 13.7% (mean ± SD, n=4 experiments) increase of cell numbers after three days in culture in comparison to culture medium alone. This growth stimulatory activity was not sensitive to heat treatment of the wound fluid (90°C, 5 minutes) but can be neutralized by addition of an antibody to porcine platelet-derived growth factor (PDGF). Our results suggest the presence of biologically active PDGF in wound fluids collected under hydrocolloid dressings. The enhancement of healing observed in wounds treated by hydrocolloid dressings may be a biological consequence of growth factor accumulation in the wound exudate.

CYTOKINE EFFECT ON CUTANEOUS T-CELL LEUKEMIA (CTCL) CELLS: SPECIFIC PROLIFERATIVE RESPONSE TO IL-4 AND IL-2. MR Charley, LJ Chan, BV Jegasothy, Dept of Dermatology, Univ of Pittsburgh.

The resistance of CTCL cells to proliferate and survive in culture has hindered the understanding of the immunobiology of CTCL. Previous human studies have shown that the only CD-4+ cells that are IL-4 responsive are those that are also CD29+. Since our previous studies have shown that all CD4+ CTCL cells are CD29+, we investigated the effect of IL-4 on purified CTCL cells. Peripheral blood cells from 2 Sezary patients were separated over Ficoll-Hypaque and yielded a > 95% pure population of CD4$^+$, CD29$^+$ cells which in each case showed a dominant T-cell antigen receptor β chain gene rearrangement in Southern analysis. Proliferative responses to solid phase x-linking of CD3 with or without added IL-1 (10 u/ml), IL-2 (10 u/ml) and/or IL-4 (50 u/ml) in all permutations were assessed by ^{3}H-thymidine incorporation and expressed as the stimulation index. Whereas normal T cells proliferated well to x-linking CD3 (Stim. Index 50X), neither CTCL population responded without added cytokines (SI 2X + 2.5X). CD3 X-linked cells from CTCL patient #1 responded well to IL-2 (SI 17X) but weakly to IL-4 (SI 3.7X); and the combination of IL-2 and IL-4 (SI 10X) was less than that of IL-2 alone. Conversely CTCL #2 responded moderately to IL-2 alone (SI 8.5X) and modestly to IL-4 alone (SI 3.5 X) but had a synergistic response to the combination (SI 23X). The addition or omission of IL-1 did not materially affect these response profiles. This in vitro proliferation system should prove useful to characterize the immunobiology of individual CTCL malignancies and to develop new immunomodulatory treatment approaches.

TRANSCRIPTIONAL ACTIVATION OF TYPE I COLLAGEN GENE EXPRESSION BY TGF-β IS INHIBITED BY IFN-γ AT POST-TRANSCRIPTIONAL LEVEL. Yue Qiu Chen, Veli-Matti Kähäri, Francesco Ramirez and Jouni Uitto. Jefferson Medical College, Philadelphia, PA; and Mt. Sinai School of Medicine, New York, NY.

Activation of type I collagen gene expression by dermal fibroblasts is an essential component of pathogenesis in dermal fibrotic diseases. Recently, the role of transforming growth factor-β (TGF-β) in this phenomenon has been emphasized. To elucidate the mechanisms involved in the activation of type I collagen synthesis, we studied the effects of TGF-β and interferon-γ (IFN-γ) on the activity of human proα2(I) collagen promoter/CAT construct in transient transfections of human skin fibroblasts and mouse NIH-3T3 cells. Exposure of transfected cells to TGF-β (0.1-10 ng/ml) resulted in a dose-dependent increase in the proα2(I) promoter activity, and the maximal stimulation (>20-fold) was obtained with 5 ng/ml. Cells incubated with TGF-β (5 ng/ml) also showed elevated type I collagen mRNA steady-state levels. Incubation of fibroblasts with TGF-β (5 ng/ml) and IFN-γ (1000 U/ml) resulted in a marked decrease in type I collagen mRNA levels compared to the cells treated with TGF-β alone. IFN-γ, however, did not affect the proα2(I) collagen promoter activity either alone or in combination with TGF-β. These results demonstrate that IFN-γ is a potent inhibitor of TGF-β induced activation of type I collagen gene expression in fibroblasts. Since IFN-γ had no effect on the transcriptional activity of type I collagen genes, the inhibiting effect takes place on the post-transcriptional level, may be mediated by decreased stability of type I collagen mRNAs.

MINOXIDIL-INDUCED CHANGES IN THE CONTRACTION OF COLLAGEN LATTICES BY HUMAN SKIN FIBROBLASTS: A NEW MEANS OF CONTROL OF EXCESSIVE CLINICAL SCAR FORMATION? George Cherry , Margaret.A. Hughes, Rodney.P.R. Dawber, and Terence.J. Ryan , Department of Dermatology, Slade Hospital, Oxford.

Previous work by Murad and Pinnell has shown that minoxidil inhibits proliferation of skin fibroblasts and lysyl hydroxylase activity. The aim of the present investigation was to study the effect of minoxidil on the contraction of hydrated collagen lattices by human dermal fibroblasts. A mixture of rat tail (Type I) collagen, sodium bicarbonate, sodium hydroxide and a cell suspension in medium containing 10% foetal calf serum and with or without minoxidil was prepared on ice, lattices cast in 6 well plates and incubated at 37°C. The rate of contraction was assessed by measuring two diameters of the lattices at right angles and calculating the percentage of initial area. Minoxidil at 10 µg/ml had no significant effect, but concentrations of 100 and 400 µg/ml inhibited contraction by approximately 10% and up to 60% respectively. Considerable inhibition was already evident within 24 hours. In contrast, the effect of minoxidil in inhibiting cell proliferation was observed even at a concentration of 10 µg/ml, but occurred only after a lapse of 48 hours. Inhibition of collagen lattice contraction was not caused by fibroblast death, since replacement of minoxidil medium by culture medium alone after several days led to rapid resumption of contraction. The clinical implication of these findings my have a role in controlling excessive scar formation.

THE YIELD OF SKIN BIOPSY IN IMMUNOCOMPROMISED CANCER PATIENTS WITH RASHES. MM Chren and CS Landefeld. Case Western Reserve University and University Hospitals, Cleveland OH.

To determine the yield of skin biopsy (BX) and the need for dermatologic consultation (CONS) in immunocompromised cancer patients with rashes, we reviewed 171 CONS for 39 months from an oncology unit. Data were gathered using a standard protocol. A BX finding was classified as major if it changed therapy. BX was done in 96 CONS (56%); no BX was done in 75 (44%), for whom follow-up showed no sequelae that would have made BX advisable. BX yielded a major finding in 21 CONS (12%), including graft-vs-host disease in 6, non-specific findings in 4, folliculitis in 3, drug-induced changes in 2, leukemia cutis in 1, vasculitis in 1, and other in 4. Major findings led to changes in systemic therapy in 13 CONS and topical therapy in 8 CONS. BX cultures were performed in 54 CONS (32%). Culture results never changed therapy, however, and disseminated infection was never established by BX or culture. Among the first 112 CONS, nonautologous bone marrow transplant and acute rash (≤2 days duration) were independent ($P<.05$) predictors of major BX findings, which occurred in 0%, 18%, and 38% of CONS with 0, 1, and 2 of the predictors, respectively ($P<.001$). In prospective testing among the next 59 CONS, however, these predictors were not related to major BX findings ($P=.6$).

We conclude that many rashes in immunocompromised cancer patients were evaluated safely without BX. Disseminated infection was never established by BX and BX cultures never changed therapy. Since clinical factors known prebiopsy are inadequate for identifying patients with major BX findings, dermatologic consultation remains critical in the evaluation of these rashes.

BASEMENT MEMBRANE AND FIBROBLAST ABERRATION IN BLISTERS AT THE DONOR, GRAFT, AND SPONTANEOUSLY HEALED SITES IN BURN PATIENTS. Bhakta V. Chetty, Raymond E. Boissy, Glenn D. Warden*, James J. Nordlund, Department of Dermatology, University of Cincinnati College of Medicine and *Shriners Hospital, Burns Institute, Cincinnati, Ohio.

Blisters which developed on spontaneously healed wounds and grafts in thirteen burn patients were analysed by light, fluorescence, and electron microscopy. Blisters developed on dermal side of dermal-epidermal junction and are more likely to develop in donor site and healed mesh graft than in split thickness sheet graft. The four major components of basement membrane zone (bullous pemphigoid antigen, laminin, type IV collagen, and epidermolysis bullosa acquisita antigen) were reduced in quantity and irregularly deposited at blister site. Immediately abjacent to the blister, epidermolysis bullosa acquisita antigen appeared normal in quantity while laminin, type IV collagen and bullous pemphigoid antigen appeared slightly reduced. Mononuclear infiltrates and autoantibodies were not detected by light microscopy or direct/indirect immunofluorescence respectively. Ultrastructurally, adjacent dermal fibroblasts demonstrated swollen rough endoplasmic reticulum and vacuolization. We speculate that blister development in burn patients is related to defective reorganization of basement membrane zone in association with dermal fibroblast aberration during wound healing.

EXPRESSION OF FIBRONECTIN AND VITRONECTIN RECEPTORS IN WOUND FIBROBLASTS. Richard A.F. Clark, James Gailit, Michael D. Pierschbacher and Erkki Ruoslahti, Departments of Medicine and Pediatrics, National Jewish Center, Denver, CO; LaJolla Cancer Research Foundation, LaJolla, CA.

Mesenchymal organization of many tissues requires fibroblast migration and extracellular matrix (ECM) assembly. Appropriate ECM receptor expression is sine que non for these processes. The β1 and β3 families of integrin membrane glycoproteins are two important classes of ECM receptors. The fibronectin receptor (α5/β1) and vitronectin receptor (αv/β3) are members of these families. In this study we have used porcine cutaneous wound repair as a paradigm of mesenchymal organization in which to correlate fibronectin and vitronectin receptor expression with fibroblast function. Polyclonal antibodies to fibronectin or vitronectin receptors isolated from human placenta or antibodies to synthetic α5 peptides were used for immunofluoresence probing of wound tissue. A fibroblast-rich granulation tissue filled approximately 80% of the wound space by day 5. Vitronectin, but not fibronectin, receptors were present on 5 day wound fibroblasts that were aligned along the wound margins and presumably migrating inward. Fibroblasts in the center of 5 day wounds were randomly oriented and expressed neither vitronectin nor fibronectin receptors. Day 7 fibroblasts were coaligned with ECM across the wound, contained cytoplasmic f-actin bundles, expressed fibronectin and vitronectin receptors, and formed cell-matrix linkages. Subsequently the wound contracted. These findings demonstrate that vitronectin but not fibronectin receptors were present during fibroblast migration into wounds, and that both receptor types were expressed coordinately with f-actin bundles and cell-matrix links just prior to wound contraction.

TRANSFORMING GROWTH FACTOR-BETA MODULATES DERMAL MICROVASCULAR ENDOTHELIAL CELL ADHESIVENESS FOR LYMPHOCYTES. Yee-Hon Chin, Vincent Falanga and Jian-Ping Cai. U. Miami School of medicine, Miami, FL.

Adhesion of leukocytes to the vascular endothelium is essential for the movements of cells from the bloodstream into inflammatory sites. Dermal microvascular endothelial cells (DMEC) isolated from normal porcine skin retain the capacity to adhere ^{51}Cr-labeled porcine peripheral blood mononuclear cells (PBMC) and T cells. The capacity of DMEC to support PBMC adhesion was increased by incubation of DMEC with recombinant cytokines such as tumor necrosis factor-alpha (TNF), gamma interferon and interleukin-1. In contrast, we found that preincubation of normal DMEC with recombinant TGF-beta (TGF-B) for 6-24 hours inhibited PBMC and T cell binding in a dose-dependent manner. Maximal inhibition was observed with a dose of 0.1 ng/ml and incubation time of 6 hours. Moreover, preincubation of DMEC with TGF-B completely blocked the capacity of these cells to respond to the enhancing effect of TNF; binding of normal lymphocytes to DMEC treated sequentially with TGF-B and TNF was significantly less than the level of binding observed for normal, untreated DMEC. Importantly, TGF-B did not affected the morphology of DMEC and there was no effect on the viability of the treated cells. In addition, the blocking effects of TGF-B can be neutralized by the addition of a polyclonal turkey anti-TGF-B antibody in the culture. These results suggested that regulation of DMEC adhesiveness is an active and dynamic process and TGF-B can potentially limit an inflammatory response by decreasing leukocyte infiltration into the sites.

SKIN IMPROVEMENT WITH A UNIQUE NEW COMPRESSION BANDAGE IN THE TREATMENT OF PATIENTS WITH VENOUS ULCERS. Chris Clay, George Cherry, Chris Cherry, Janice Cameron, and Terence Ryan, Department of Dermatology, Slade Hospital, Oxford, U.K.

Adequate compression bandaging to reduce lower leg venous hypertension is the most important aspect of the treatment of ambulatory patients with venous ulceration. Conventional methods of providing compression such as Unna boots have been used for many years but are associated with a number of problems. An ideal bandage would be one that provides adequate gradient compression as well as a local environment which is conducive to surrounding skin improvement. For the last year we have evaluated a new adhesive compression bandage (DuoDerm Adhesive Compression Bandage) with a hydrocolloid lining that meets the above criteria. In ten patients with venous ulcers silicone replicas were made of the surrounding skin prior to application and at each change of the new bandage. The replicas were studied at low magnification with scanning electron microscopy (SEM) and graded with the Beagley-Gibson system for skin quality. The gross appearance of the skin was markedly improved with scale removal and reduction of hyperkeratosis as early as 24 hours after application of the bandage. The SEM studies revealed similar improvement in the state of individual skin cells in all of the patients. Flaky cells seen before application of the compression bandage were lost and were replaced by flat squamous cells with tightly opposed cell junctions similar to those seen in normal skin.

Thus in summary these studies demonstrate that this new hydrocolloid-lined compression bandage that provides adequate support to reduce venous hypertension in patients with venous ulcers also improves the gross appearance of the skin as well as the ultrastructural integrity.

MORPHOLOGY OF PITYROSPORUM OVALE AND ITS LOCATION ON HUMAN SCALPS IN THE DANDRUFF CONDITION. Don W. Coble, Mary Bell, Anne E. Maczulak, and Charles W. Slife, Gillette Research Institute, Gaithersburg, MD.

The human scalp is inhabited by aerobic bacteria, the anaerobe Propionibacterium, and the yeast Pityrosporum. On dandruff scalps, Pityrosporum is the most abundant of these organisms and may play an important role in the condition. To further investigate this role, a morphological examination of the yeast was carried out. Scurf was collected from nineteen individuals with dandruff and examined by scanning electron microscopy. Pityrosporum cells were chiefly found in clusters together with keratinocytes and were easily identified by their bottle-shaped appearance and collarette which is a unique feature of the organism. The same features were observed in Pityrosporum cultures, as was a new feature, a continuous ridge which spirals around the yeast up to 14 times. This ridge appears to be contiguous with another ridged structure found on the inner surface of the cell wall. The location of the yeast on the scalp was determined by examining scalp biopsies with transmission electron microscopy. Pityrosporum cells were readily found on the surface of the scalp, especially near the outer infundibulum. Interestingly, they also occasionally were observed in the hair follicle as much as 400 microns below the scalp surface. The presence of the yeast in the canal where the organism is exposed to a different environment than on the surface may be important to the dandruff condition.

MECHANISMS OF T SUPPRESSOR CELL GENERATION BY HUMAN UV-EXPOSED EPIDERMAL CELLS. KD Cooper, O Baadsgaard, B Salvo, and D Fox. Immunodermatology Unit, Dept. of Dermatology, and Div. of Rheumatology, Univ of Michigan, Ann Arbor, MI.

Human epidermal cells(EC) obtained 3 days after 3 MED UV exposure in vivo contain OKM5(CD36)$^+$ macrophages which activate autologous CD4$^+$ T cells in the absence of exogenous antigens. These CD4$^+$ T cells profoundly suppress Pokeweed Mitogen(PWM)-driven blood mononuclear leukocyte(MNL) IgG production. To characterize how sunburned skin activates immune suppressive mechanisms, we used various antigen presenting cell populations(APC's) to activate autologous CD4$^+$ subsets prior to their addition to PWM-stimulated MNL. Only when UV-exposed EC (UV-EC) were used to stimulate CD4$^+$ T cells containing the CD45R$^+$ suppressor-inducer subset was significant suppression of IgG production by PWM-stimulated MNL observed (500 ng IgG); removal of CD45R$^+$ lymphocytes from CD4$^+$ T cells resulted in depletion of suppressive activity (1100 ng IgG) in 3/3 experiments. UV-exposed EC, relative to control EC or blood APC enriched for dendritic cells, demonstrated enhanced ability to activate T suppression; this difference could not be attributed simply to differences in the degree of T cell activation. Thus, CD4$^+$ T cells proliferated equally in response to the Tetanus Toxoid(TT) antigen when presented by UV-exposed EC(5372 delta cpm ^{3}H-TdR uptake) or blood APC (2809 delta cpm ^{3}H- TdR). However, the T cells activated by UV-EC and TT were suppressive(3631 ng IgG) while the T cells activated by blood APC and TT provided help(47863 ng IgG), relative to unregulated PWM-MNL(30903 ng IgG). Although the above TT-responsive CD4$^+$ T cells failed to override UV-induced suppression, suppression induced by CD4$^+$ T cells activated by UV-EC (in the absence of added antigen) (2754 ng IgG) was reversed by the addition of normal blood APC to UV-EC and CD4$^+$ T cells(20893 ng IgG). Thus, in this in vitro human model of UV-induced immunosuppression, UV-EC provide signals that preferentially activate CD4$^+$ T suppressor-inducer cells. The signals delivered by UV-EC generate suppression even when non UV antigen-responsive T cells are activated, but these signals are blocked by normal blood APC, suggesting counterbalancing forces.

GENOMIC LOCALIZATION OF MOUSE HAIR KERATIN GENES. John G. Compton and Arthur P. Bertolino, The Jackson Laboratory, Bar Harbor, ME, and Hair Disease Research Laboratory, Epithelial Biology Unit, Department of Dermatology, NYU Medical Center, New York, NY.

Little is known about the genomic organization and regulation of the hair keratin gene family. Like the epithelial cytokeratins, the hair keratin genes (at least 10) fall into type I and type II subfamilies. Cloned and sequenced mouse hair keratin cDNAs MHKA-1, MHKB-1 and MHKB-2 provided specific probes for investigating hair keratin gene organization. Since the hair and epithelial keratins are homologous in their α-helical rod domains, our mapping focused on the known chromosomal organization of mouse cytokeratins into only two clusters of homologous genes, the Krt-2 locus of type II genes on Chr. 15, and the Krt-1 locus on Chr. 11. These keratin loci are tightly-linked to mouse mutations affecting mouse skin and hair, Re, Den, and Bsk on Chr. 11, and Ve, Sha, Ca and N on Chr. 15. DNA from the progeny of C57BL/6J x M. spretus backcrossed mice were typed for segregation of hair keratin alleles, Krt-1 and Krt-2 alleles and other closely-linked genes. No recombination was observed between MHKA-1 and Krt-1, or between Krt-2 and either MHKB-1 or MHKB-2. These mapping results suggest that most, if not all type I and type II hair keratin genes are part of the Krt-1 and Krt-2 loci, repsectively. The non-dispersion of homologous keratin genes in the mammalian genome leads us to hypothesize that a domain organization of the genes has influenced evolution of the keratin gene family, and may play a significant role in tissue-specific and developmental regulation of keratin gene expression as well.

AN ANIMAL MODEL FOR THE STUDY OF GIANT CONGENITAL NEVOMELANOCYTIC NEVI Matthew Cooper, Richard Spielvogel*, John Hansbrough, Steven Boyce, Tanya Foreman, and David Frank. Dept of Surgery, UCSD Medical Center, San Diego, CA and *Dept of Dermatology, Hahnemann Univ., Phil., PA.

This study addresses the development of an animal model for human giant congenital nevomelanocytic nevi (GCNN), employing a dermal-epidermal composite cultured skin substitute and the athymic mouse.

Grafts were made from: 1) non-involved split-thickness skin (STS) from 12 month old GCNN patient, 2) nevus STS from same GCNN patient, 3) nevus full-thickness skin (FTS), and 4) cadaveric human STS. For groups 1) & 2), human keratinocytes (HK) and fibroblasts (HF) were enzymatically isolated, expanded in tissue culture, and composite grafts were made by placing HF into a collagen-GAG matrix, and HK inoculated to the non-porous surface. Groups 3 & 4 were not cultured.

Full-thickness (2 X 2 cm) wounds were created on the athymic mice and were covered with grafts from each of the four groups. Biopsies were obtained from 6 to 38 weeks post placement for elastic tissue, S-100 immunoperoxidase, and light and electron microscopy.

The GCNN cultured skin mice (group 2) developed black, raised skin in the healed wounds. None of the group 1 mice developed lesions, grossly or histologically. All of the nevus FTS mice retained the nevus grossly. Histopathologic examination at 38 weeks of the black, raised plaques of group 2 demonstrated a reconstituted dermis similar to group 3. Nevus cells were larger and more epithelioid in the upper dermis, as seen with true GCNN. The melanocyte identity of these cells was confirmed with S-100 staining and electron microscopy.

These findings are unique to this cultured graft system and provide a new basis for the study of GCNN and melanocyte biology in vivo.

VITAMIN A DEFICIENCY AND EPIDERMAL RESPONSE TO TUMOR-PROMOTERS. Michael J. Connor, Division of Dermatology, UCLA School of Medicine, Los Angeles, California.

Exogenous retinoids can modulate skin-carcinogenesis and the induction of epidermal ornithine decarboxylase by tumor promoters. We have examined the role of endogenous retinoids in this process, by determining the impact of vitamin A deficiency on the induction of epidermal ornithine decarboxylase. To obtain vitamin A deficient mice, gestating female *skh/hr1* hairless mice were fed test diets [containing 0 (low), 400 IU/kg (marginal), or 4000 IU/kg (high) vitamin A] one week prior to parturition and during nursing, and the young were reared on the same diets. Vitamin A status was established by determining liver and cutaneous retinoids; by monitoring body weights and food consumption; and by histological examination of mucus-secreting epithelia for signs of abnormal keratinization. These parameters confirmed that the three test diets produced severely vitamin A depleted, marginally vitamin A depleted and vitamin A replete groups of mice respectively. As observed in other studies, vitamin A deficiency produced marked tracheal keratinization but had no significant impact on cutaneous histology. Mice from each group were treated topically with the tumor promoter tetradecanoyl-phorbol-13-acetate (10 nmol) to induce epidermal ornithine decarboxylase. The level of ornithine decarboxylase activity induced was found to be diet dependent. Activities in mice fed the high vitamin A diet were similar to those fed standard rodent chow. Activities were 2-3 fold higher in the marginally vitamin A deficient mice compared to mice fed the high vitamin A diet. In contrast, the ornithine decarboxylase activity induced in mice fed the severely deficient diet was lower than in mice fed the marginally deficient diet. These results suggest that endogenous vitamin A is required for ornithine decarboxylase induction, and supports the proposal that physiological amounts of vitamin A are required for tumor-promotion. Moderate rather than severe vitamin A deficiency may be a greater risk factor for skin carcinogenesis.

IDENTIFICATION OF EXTRACELLULAR MATRIX MOLECULES IN DERMAL PAPILLA DURING HAIR FORMATION AND THE HAIR CYCLE. E. Jill Copeland and Steven Ledbetter, The Upjohn Company, Kalamazoo, MI.

We have examined the expression of extracellular matrix proteins and proteoglycans, using monospecific antibodies, in the formation of hair follicles and during the cycle of mature follicles in mouse skin. We find that the neural cell adhesion molecule (N-CAM) and the proteoglycan Syndecan are early markers of the condensed mesenchyme prior to epithelial downgrowth in embryonic mouse skin. Syndecan remains localized to presumptive dermal papilla until epithlial downgrowth is complete at which time it localizes only to the epithelium. Tenascin and its binding proteoglycan are expressed later in embryonic papillae. In mature pelage hair, N-CAM and the basement membrane heparan sulfate proteoglycan localize to dermal papillae throughout the hair cycle and in epithelial basement membranes. Syndecan is expressed by hair epithelial cells throughout the cycle and is reexpressed by papilla cells in very early anagen before epithelial contact is reestablished. Tenascin and its binding proteoglycan both localize to dermal papillae in catagen and early anagen, however tenascin expression is lost before the proteoglycan. We have provided evidence that dermal papilla cells produce an extracellular matrix that is distinguished from the dermis, that there is similarity with matrix produced by neural crest cells and finally, the composition of dermal papillae matrix produced during hair formation is not strictly comparable to the matrix produced in mature follicles.

EFFECT OF NUCLEOTIDES ON CYTOSOLIC FREE CALCIUM IN INDIVIDUAL CANINE KERATINOCYTES. Flavio M. Crameri, Maja M. Suter, James P. Slattery, and Paul J. Millard, Departments of Pathology and Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

Changes in intracellular free calcium ($[Ca^{2+}]_i$) play an important role in a variety of biochemical reactions that lead to cellular responses such as proliferation and differentiation. The response of $[Ca^{2+}]_i$ to extracellular ATP, UTP, and ITP was determined in individual canine keratinocytes using the fluorescent probe fura-2 and digital video fluorescence imaging microscopy. Subconfluent cultures were grown for 48 hours on glass coverslips in William's medium E with 10ng/ml EGF, 10^{-10} M cholera toxin, 10% fetal bovine serum (complete WME). Fifteen hours prior to the experiments the cells were switched to WME only.

In the presence of 1.8 mM extracellular Ca^{2+}, 100 and 500 μM ATP caused a rapid (< 9 sec) 3- to 10-fold rise in $[Ca^{2+}]_i$ above resting levels of 50-100 nM followed by occasional fluctuations. Small responses were elicited with doses as low as 0.1 μM ATP. Cells kept in complete WME up to the time of experiment did not respond. The response of cells stimulated with 500μM ATP in Ca^{2+}-free medium was characterized by a 1.5- to 3-fold rapid initial peak followed by a decrease of $[Ca^{2+}]_i$ below resting levels. Loss of response occurred in the majority of keratinocytes preincubated for 2 min in Ca^{2+}-free medium. UTP was as effective as ATP and a small but significant elevation in $[Ca^{2+}]_i$ was noted with 500μM ITP.

These results suggest that ATP-induced initial rises of $[Ca^{2+}]_i$ may be enhanced by extracellular Ca^{2+} and that sustained rises are completely dependent on extracellular Ca^{2+}. Intracellular Ca^{2+} stores appear to be rapidly depleted after addition of Ca^{2+}-free medium.

LOW EXPRESSION OF ICAM-1 IN THE DEPIGMENTING C57BL/LER-VIT/VIT MICE: THE PROBABLE CAUSE FOR LOSS OF CONTACT SENSITIZATION. Miklos Csato, *Fumio Takei, James J. Nordlund, Dept Dermatology, Univ of Cincinnati College of Medicine, Cincinnati, OH, and *Terry Fox Laboratory, BC Cancer Research Center, Vancouver, BC, Canada.

The depigmenting C57BL/Ler-vit/vit (vit/vit) mouse, a congenic mutant of the C57BL/6 strain exhibits a single immune deficiency, an inability to respond to epicutaneous application of DNFB or TNCB. The aim of our study was to delineate the cause for this deficiency. Epicutaneous application of the hapten TNCB and subcutaneous (s.c.) injection of hapten (TNBS)-modified C57BL/6 as well as vit/vit epidermal cells (EC) sensitized all congenic C57BL/6 animals. This suggests vit/vit EC can present the antigen. In the vit/vit mice, however, only the s.c. injection of haptenized EC caused sensitization. Sensitization by s.c. injected haptenized EC was documented by adoptive transfer of splenic lymphocytes into naive C57BL/6 animals which then reacted to challenge doses of TNCB. This suggests vit/vit lymphocytes can react to TNCB antigen. Vit/vit lymphocytes did not react in allogeneic mixed epidermal cell-lymphocyte reaction, but mounted a normal response in syngeneic reaction. These findings suggested a possible adhesion signal deficiency in the vit/vit mice. Since ICAM-1 is the best characterized epidermal adhesion molecule, we studied its expression in the vit/vit epidermis using the YN1/1 monoclonal antibody against MALA-2, the murine counterpart of human ICAM-1. The vit/vit epidermis did not stain with the antibody before or after challenge with the hapten. Vit/vit EC expressed less ICAM-1 upon stimulation with IFN-γ or TPA *in vitro*. Adherence of vit/vit lymphocytes was lower to IFN-γ or TPA treated vit/vit than to C57BL/6 EC. We conclude that the loss of contact sensitivity results from an abnormal EC/T-cell interaction accounted for by low expression of ICAM-1 in the vitiligo animals.

THE 5'-UPSTREAM REGION OF CYTOKERATIN K19 CONTAINS A SEQUENCE ELEMENT THAT IS TRANSCRIPTIONALLY ACTIVE AND BINDS TO A TRANSCRIPTION FACTOR. James F. Crish and Richard L. Eckert, Departments of Dermatology and Physiology and Biophysics, Case Western Reserve University Medical School, Cleveland, Ohio.

In recent years a great deal of new knowledge has been gained regarding keratin structure. However, less is known regarding the mechanisms that regulate cytokeratin gene expression. With a goal of gaining new information regarding these mechanisms, we have identified a sequence in the upstream region of cytokeratin K19 that binds transcription factor(s). A ^{32}P-labeled oligonucleotide (26 mer) containing the factor binding site shifts a single band in gel shift assays. The band is specifically competed by 40- and 400-fold excess of the identical radioinert oligonucleotide but not by heterologous oligomers. The binding activity is found in a wide variety of epithelial cells and, interestingly, is competed by other oligonucleotides, previously identified as binding to the CCAAT family of transcription factors.

This region also displays functional activity in transcription assays. The element drives 4- to 10-fold higher levels of chloramphenicol acetyltransferase activity (CAT) when cloned into a minimal promoter/CAT reporter plasmid compared to a control plasmid lacking the element.

The CCAAT family of proteins are known to be general transcription factors that interact with a variety of genes. Recent evidence suggests that general and specific factors form multi-peptide complexes to activate transcription. It is possible that other factors associate with regions adjacent to this element to mediate tissue specific expression.

ELASTIC PROPERTIES OF HUMAN SKIN: RELATION TO AGE, SEX AND ANATOMICAL REGION. Anastasia B. Cua, Klaus-P. Wilhelm, and Howard I. Maibach, Department of Dermatology, University of California, San Francisco.

Using a recently developed non-invasive, in vivo suction device for measuring skin elasticity, we evaluated age, sex and regional differences in the viscoelastic properties of skin. A total of 33 volunteers participated in the study consisting of: a) 8 young females, b) 9 old females, c) 8 young males and d) 8 old males. Measurements were performed on 11 anatomical regions by application of vacuum perpendicular to the skin surface; three different loads were applied: 100, 200 and 500 mbar. The parameters used were: Ue, immediate distension; Uv, delayed distension; Ur, immediate retraction, and Uf, final deformation. To compare between subjects and anatomical regions, relative parameters independent of skin thickness were calculated: Uv/Ue, the ratio between the viscoelastic properties of skin and immediate distension and Ur/Uf, which measures the ability of the skin to regain its initial position after deformation. Generally, Uv/Ue increased while Ur/Uf decreased with aging. Responses were variable with respect to load applied. Variability within anatomical regions was also noted. However, differences between the sexes were not statistically significant for most regions. These findings are in agreement with earlier studies suggesting the differences are mainly attributable to alterations in the equilibrium of the "ground substance" of the dermis and changes in the elastic fiber network.

Precluding invasive techniques such as biopsy, this procedure provides simple, quantitative assessment of elastic properties of skin, its use of which may help in future investigations and evaluation of pharmacologic modulations for other connective tissue disorders.

IMMUNODOMINANCE, STRAIN-CHARACTERIZATION, AND MHC-RESTRICTION OF THE UNRESPONSIVENESS INDUCED BY UVB-IRRADIATED LANGERHANS CELLS (LC). P.D.Cruz,Jr.,R.E.Tigelaar,P.R.Bergstresser, UT Southwestern,Dallas,TX.

Low-dose UVB exposure leads to suppression of contact sensitivity (CS) in some but not all mouse strains; LC have been implicated as inducers of this unresponsiveness. In this study, we determined whether: 1) the capacity of LC to induce CS or of irradiated LC to initiate tolerance is dominant, 2) susceptibility to UVB-suppression is related to LC genotype and 3) self-recognition is involved in this unresponsive state. The ability of LC from susceptible (C3H/HeN) and resistant (BALB/c) strains to induce and regulate CS following TNP-haptenation and intravenous syngeneic transfers was compared. In both strains, mice infused with TNP-LC developed CS indistinguishable from those of conventionally sensitized mice, whereas mice injected with low-dose UVB (200 J/m^2)-irradiated TNP-LC displayed down-regulated responses. Cotransfer of equal numbers of TNP-LC and UVB-TNP-LC into syngeneic mice also resulted in down-regulated responses. A requirement for MHC identity was identified using allogeneic transfers: UVB-TNP-LC from either BALB/c (Iad) or C3H/HeN (Iak) failed to induce tolerance when infused into the allogeneic strain. Finally, syngeneic transfers into mice treated with anti-CD8 or anti-CD4 Ab revealed CS from TNP-LC infusion to be mediated by both CD4$^+$ and CD8$^+$ T cells, whereas the tolerance induced by UVB-TNP-LC was mediated primarily by CD4$^+$ T cells. We conclude that low-dose UVB confers upon LC the capacity to initiate unresponsiveness in a MHC class II-restricted manner; once evoked, this attribute is dominant and appears to be functional in both UVB susceptible and resistant strains.

ANALYSIS OF PIGMENT CELL ANTIGENS DEFINED BY VITILIGO ANTIBODIES. J Cui, R Harning, J Li, J-C Bystryn, Dept of Dermatology, NYU School of Medicine, New York, N.Y.

The etiology of vitiligo is not known although there is evidence that autoantibodies are important in its pathogenesis. We measured the presence and specificity of antibodies to pigment cells in 23 pts with vitiligo and in 22 control pts with unrelated conditions using as an assay immunoprecipitation of 125-I labelled cell surface antigens on melanoma cells followed by SDS-PAGE and autoradiography analysis. Antibodies to melanoma antigens were present in 18 (78%) pts with vitiligo but in only 3 (12%) control pts ($p<0.05$). The antibodies were directed to one or more cell surface antigens with MWs of approximately 35, 40, 45, 75, 90 or 150 kDs. The responses were most commonly directed to the 40 kD antigen (in 74% of pts), then to the 70 kD, 45 kD, and 90 kD ags (antibodies present in 57%, 35%, and 30% of pts respectively). Most of these antigens were also expressed on one or more control cell lines, indicating they were not specific for pigment cells.

These results confirm that antibodies to pigment cells are more common in pts with vitiligo, and indicate that they are directed to several different antigens which do not appear to be pigment cell specific. Consequently, the selective killing of melanocytes in vitiligo may be due to the greater sensitivity of these cells to immune injury.

IN SITU VISUALIZATION OF THE LAMELLAR BODY SECRETORY SYSTEM BY CONFOCAL MICROSCOPY. Christopher Cullander*, Gopinathan K. Menon†, Richard H. Guy*, Peter M. Elias† *Depts. of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy and †Dept. of Dermatology, School of Medicine, University of California, San Francisco, CA .

Laser-scanning confocal microscopy (LSCM) can be used to optically section tissues that are sufficiently: a) transparent; b) thin (to avoid loss of resolution); and c) free of interfering autofluorescence. Moreover, fresh tissues can be studied without prior fixation and embedding - a particular advantage for lipids, which are preferentially lost through processing for light or electron microscopy. To determine whether LSCM could be used to visualize the distribution and fate of epidermal lamellar bodies, we obtained intact sheets of neonatal mouse stratum corneum (SC) and stratum granulosum (SG) two hours after intracutaneous injection of purified staphylococcal epidermolytic toxin. These sheets fulfill the above criteria of transparency, thinness, and lack of autofluorescent structures, such as pilosebaceous lipids. After application of Nile Red (in glycerol, prepared as per [1]), which fluoresces after binding to non-polar lipids, the distribution of lamellar bodies within the SG, followed by their deposition into the intercellular spaces at the SG-SC interface, could be clearly visualized by LSCM (excitation = 514 nm, and emission >550 nm). Control full-thickness epidermal preparations, prepared with 10 mM EDTA, did not reveal comparable structures in the basal or spinous layers, but again Nile Red-positive structures, corresponding to lamellar bodies, appeared in the SG. Therefore, laser-scanning confocal microscopy offers a new way to study the dynamics of lamellar body secretion under normal and experimentally perturbed conditions.

[1] Fowler and Greenspan, J. Histochem. Cytochem. 33:833, 1985.

THE BINDING AND INTERNALIZATION OF EXOGENOUS DNA IN HUMAN LYMPHOCYTES. R. Dall'Amico, M. O'Malley, R. Edelson, and F. Gasparro. Department of Dermatology, Yale U., New Haven, CT.

To determine the functional role and significance of DNA associated with the cell membranes of normal human lymphocytes, we have examined the kinetics of DNA internalization and its subsequent incorporation into nuclear DNA. Fluorescence microscopy showed that sonicated rhodamine-labeled DNA is bound and internalized by PHA-stimulated lymphocytes. This process is detectable as early as 1 h following the addition of the DNA and continues over a 48 h period. Treatment with 8-MOP plus UVA decreased the ability of lymphocytes to internalize DNA. At 20 ng/ml 8-MOP and 5 J/cm^2 UVA, this decrease was 40% and at 100 ng/ml and 5 J/cm^2, 73%, compared to control samples treated with either 8-MOP or with UVA. To determine the fate of this internalized DNA, PHA-stimulated lymphocytes were exposed to sonicated [^{32}P]-labeled DNA. At various times, cells were collected and the nuclei from the cells were isolated. Analysis of these nuclear fractions revealed an increase in the counts per million cells: 532 cpm at 6 h; 1468 cpm at 24 h; and 2496 cpm at 48 h (25% of the total counts bound by the cells). DNA extracted from these samples was analyzed on a polyacrylamide gradient gel (5-20%). At 6 h autoradiography showed a signal of increasing intensity. The sizes of the DNA fragments were much larger than the original DNA added to the cultures. Thus, exogenous DNA enters the nuclei of cultured lymphocytes and is incorporated into newly synthesized DNA.

REDUCED EXPRESSION OF CELL ADHESION MOLECULE UVOMORULIN IN PROLIFERATIVE SKIN DISORDERS. W Czech*, J Krutmann*, K Herrenknecht*, A Kapp*, E Schöpf*, *Dept. Derm., Univ. Freiburg; *Max-Planck-Institute for Immunobiology, Freiburg, FRG.

The mouse cell adhesion molecule (CAM) uvomorulin plays an important role during embryonic development and in the maintenance of adult epithelia. Uvomorulin is evolutionary highly conserved and antibodies recognize the human homologue. We have studied uvomorulin expression in normal human skin and several proliferative skin lesions using affinity purified antibodies in immunoflourescence tests. In normal human epidermis (n=10), basal and suprabasal keratinocytes showed a strong and homogenous cell membrane staining. The cell membrane part of keratinocytes which faces the basal lamina was negative. However uvomorulin expression was significantly decreased in squamous cell (n=5) as well as in solid basal cell carcinoma (n=5). The latter showed a dimorphic staining with a reduced flouresence of the inner cell layers and normal staining at the periphery. Decreased uvomorulin expression was not specific for malignant skin lesions, since it could also be observed in condyloma acuminatum. These studies demonstrate that uvomorulin is decreased in proliferative skin disorders. Differential expression of this CAM may account at least in part for the differences in clinical course between squamous cell and basal cell carcinoma.

THE DIFFERENTIAL REGULATION OF PDGF-A AND PDGF-B IN NORMAL HUMAN KERATINOCYTES. D. Damm*, G. Shipley#, C. Hart†, and J. Ansel*, *Dermatology Service, VA Medical Center, #Department of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR, and †Zymogenetics Corp., Seattle, WA

PDGF is a polypeptide growth factor which is a potent mitogen for a number of connective tissue cells such as dermal fibroblasts, smooth muscle cells and glial cells. It is also chemotactic for monocytes, neutrophils, and fibroblasts. Because of these properties it could be an important mediator in wound healing and inflammatory processes in the skin. We have recently reported for the first time that normal human keratinocytes can express PDGF mRNA. In this study the modulation of PDGF-A/PDGF-B and the expression of PDGF receptors in normal human keratinocytes were examined. Our results indicated that (1) normal human keratinocytes express and secrete PDGF, (2) IL-1α and TGFß differentially modulate human keratinocyte PDGF A&B mRNA, (3) uninduced human keratinocytes predominantly express PDGF-A mRNA and secrete PDGF-AA, whereas keratinocytes induced by IL-1α and TGFß express mostly PDGF-B mRNA and (4) normal keratinocytes do not express either PDGFα or ß receptors and thus can not respond to this factor. Since dermal fibroblasts respond poorly to PDGF-AA but significantly to PDGF-AB and PDGF-BB, these results may have important biological implications; with uninduced keratinocytes producing an isotype of PDGF with little cutaneous bioactivity (PDGF-AA), but induced keratinocytes producing PDGF isotypes (PDGF-AB, PDGF-BB) with significant cutaneous bioactivity. Studies in our laboratory examining the production of the various isotypes of keratinocyte PDGF in normal and diseased skin will help address this issue.

CHILD SYNDROME: LACK OF EXPRESSION OF EPIDERMAL DIFFERENTIATION MARKERS. B.A. Dale, J.R. Kimball, P. Fleckman, K.A. Holbrook, A. Hebert, Univ of Washington, Seattle, WA & Univ. of Texas, Houston TX

Congenital Hemidysplasia Ichthyosiform with Limb Defects (CHILD) syndrome is a rare genetic disorder. Biopsies from affected and unaffected plantar skin from a 3 year old patient were analyzed for expression of keratins and filaggrin by biochemical and immunohistochemical methods, for ultrastructural abnormalities, and for growth of epidermal keratinocytes. The affected plantar epidermis was parakeratotic and 3-4 times thicker than unaffected, showing reduced numbers of keratohyalin granules and keratin filaments. In unaffected plantar epidermis, basal cell keratins, K5/14, were a small fraction of total keratins, while suprabasal keratins, K1/10, were strongly expressed and the proteolytically processed K1 was prominent. Profilaggrin and filaggrin and the 60 kDa keratin, a differentiation marker specific for sole, were also present. In contrast, in the affected plantar epidermis, expression of all differentiation markers was significantly reduced or absent and keratin K1 was not proteolytically processed; hyperproliferative keratins, K6/16, were strongly expressed. Increased expression of K5/14 compared to the unaffected sample suggested that basal cell keratin expression was not down-regulated as in normal epidermis. Keratinocytes from both unaffected and affected biopsies, grown in culture, had similar morphology and keratin and profilaggrin expression, in contrast to the extreme differences in situ. Thus, systemic or dermal factors are important components of the abnormal ichthyosiform, epidermal differentiation seen in CHILD syndrome.

UV-B IRRADIATION OF NORMAL HUMAN FORESKIN MELANOCYTES IN CULTURE. Athena Daniolos, Ruth Halaban, Aaron B. Lerner, Michael R. Lerner, Dept of Dermatology, Yale Univ. School of Medicine, New Haven, Connecticut.

Solar ultraviolet radiation induces numerous photobiologic effects in the skin via poorly understood mechanisms. In melanophores of lower vertebrates (Xenopus laevis), light (350-525nm) induces a prompt intracellular increase in cAMP levels, and a visible dispersion of melanosomes.

To investigate whether changes in intracellular second messengers occurred in normal cultured human foreskin melanocytes following UV-B irradiation, 3', 5'-cyclic adenosine monophosphate levels were measured with a Rianen cAMP RIA kit. Following a 24 hr. incubation in defined PC-1 medium containing basic fibroblast growth factor (bFGF, 1ng/ml), UV-B irradiation with 20 mJ/cm^2 induced a rapid, 2-fold increase in intracellular cAMP levels. This UV-B induced elevation in c-AMP was not seen in similarly irradiated human keratinocytes or fibroblasts.

THE lpr GENE IS NOT A PREREQUISITE FOR SKIN DISEASE IN THE MRL MOUSE. Kathleen M. David, Michiyo Tomita-Yamaguchi, Thomas J. Santoro, and Lela A. Lee, Depts of Dermatology and Medicine, U. of Colorado and Denver VA, Denver, CO.

The MRL-*lpr/lpr* mouse has been studied as an animal model of systemic lupus erythematosus. These mice develop antinuclear antibodies (ANAs), arthritis, glomerulonephritis, skin lesions, and massive lymphoproliferation. The skin lesions have been studied by Furukawa et al., who found hyperkeratosis, acanthosis, vacuolar alteration at the dermal-epidermal junction (DEJ), vascular dilatation, extravasated red blood cells (RBCs) in the upper dermis, and a mononuclear dermal infiltrate, findings similar to hyperkeratotic discoid lupus. The *lpr* gene, which causes massive lymphoproliferation, has been believed to be responsible for the lupus-like disease.

The MRL-+/+ mouse is a congenic inbred strain which appears to have at least 96% of genomes in common with the MRL-*lpr/lpr* mouse and to be without the autosomal recessive mutant *lpr* gene. Therefore, the MRL-+/+ mouse does not develop massive lymphoproliferation. In addition, ANAs and kidney disease are delayed in onset. Skin lesions have been thought to occur only rarely in the MRL-+/+ and have not been characterized. We report that skin lesions occurred in 121 of 142 (85%) female and in 39 of 82 (48%) male MRL-+/+ mice 28 weeks of age or older. Lesions ranged from erythema to crusted inflammatory lesions to scarring alopecia to autoamputation. Lesions occurred primarily on the snout, ears, back, and tail. Within sibling groups, the type of lesion and sites affected were quite homogeneous. For example, some sibling groups had snout alopeica only, while others had autoamputated tails but no snout involvement. In 15 of 16 MRL-+/+ mice examined with routine histology, lesional skin showed hyperkeratosis, acanthosis, vacuolar alteration at the DEJ, vascular dilatation and extravasated RBCs in the upper dermis, and a mononuclear dermal infiltrate. These findings suggest that the *lpr* gene is not required for the development of skin lesions in MRL mice. Further, this data substantiates the hypothesis that the *lpr* gene and massive lymphoproliferation are not required for the development of lupus-like disease in MRL mice.

AN IN VITRO ALTERNATIVE TO PRIMARY DERMAL IRRITANCY ASSAYS. V. DeLeo, J. Hong, G. Lamont, B. Kong, S. DeSalva, Department of Dermatology, Columbia University, New York, NY, and Colgate-Palmolive, Piscataway, NJ.

Current premarketing testing of consumer products for safety requires animal assays for dermal irritancy. In vitro assays would be both humane and cost effective. We have previously shown that surfactants induce the release of arachidonic acid (AA) from prelabelled mammalian cells in culture and this release relates positively to animal skin irritancy. We studied the effect of 27 finished products (FP) on AA release. C3H10T1/2 cells were cultured; prelabelled with [3H]AA; and treated with selected dilutions of FP, with media alone as control. An Irritant Dilution 200 (ID200) was determined for each product by combining a number of assays and generating a best fit line. ID200 represents the dilution of FP necessary to induce release of [3H]AA twice control values. When the ID200 for each of the 27 FP were compared to the animal irritancy score for each product (PDII - Primary Dermal Irritancy Index), the correlation using both Pearson and Spearman-Rho tests was significant at $p<0.0314$ and $p<0.025$, respectively. When agents of extreme pH were excluded the correlation between ID200 and PDII improved to $p<0.0084$ and $p<0.01$, N=25 FP; and to $p<0.0001$ and $p<0.001$, N=19 FP, with the added exclusion of insoluble outliers. These data suggest that ID200 determination holds promise as a non-animal assay for dermal irritancy testing.

CLONING AND CHARACTERIZATION OF THE TRANSCRIPTIONAL REGULATORY REGION OF THE HUMAN INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) GENE. K. Degitz, L.-J. Li, and S.W. Caughman, Dermatology Branch, National Cancer Institute, NIH, Bethesda, MD.

The expression of ICAM-1, a specific ligand for the leukocyte adherence molecule LFA-1, by human keratinocytes (HK) and dermal endothelial cells is regulated by various cytokines, including interferon (IFN)-γ, and is of critical importance in the evolution of inflammatory processes in the skin. To elucidate the transcriptional regulatory mechanisms involved in ICAM-1 expression, the genomic structure of human ICAM-1 including the 5' flanking regulatory region was determined and analyzed. Southern blot analysis of human genomic DNA using various portions of an ICAM-1 cDNA indicated that the ICAM-1 gene was larger than 15 kb. A 17 kb fragment from the 5' region of the ICAM-1 gene was isolated by screening a human lymphocyte λ phage genomic library with a 315 bp 5' fragment of the ICAM-1 cDNA. Restriction enzyme mapping, Southern blot analysis and sequencing of this genomic clone revealed that it contained sequences corresponding to the first 794 bp of the ICAM-1 cDNA, and that these sequences were divided among three exons dispersed over 12.5 kb of genomic DNA. A 2.05 kb Eco RI subclone, containing the 5'-most exon and an additional 1335 bp upstream, was sequenced and analyzed for transcription initiation (TI) sites and promoter/enhancer elements. Primer extension reactions, utilizing radiolabeled primers complementary to various portions of ICAM-1 mRNA and templates consisting of both total cellular and poly-A enriched RNA from IFN-γ treated HK and A431 cells, identified a consistent TI site 55 bp 5' of the ICAM-1 translation start site. Analysis of the 5' flanking region and portions of the first intron revealed an appropriately placed TATA box, as well as consensus binding sequences for the transcriptional activating factors induced by IFN and for SP1, AP-1, AP-2, glucocorticoid receptor, and NFκB. These findings make possible detailed molecular studies of the transcriptional regulation of ICAM-1 by cytokines and other factors.

COMPARATIVE EFFECTS OF CsA, TGF_B, AND CHLOROQUINE ON ALLOANTIGEN PRESENTING CELLS OF BLOOD AND EPIDERMIS. Aicha Demidem, J. Richard Taylor, and J. Wayne Streilein. Departments of Microbiology/Immunology, and Dermatology, University of Miami School of Medicine, Miami, Florida.

Antigen presenting properties of epidermal cells (EC), including Langerhans cells (LC), are abnormal in psoriatic skin, and resemble the properties of peripheral blood mononuclear cells (PBM). To investigate the possibility that antigen presenting cells (APC) might be involved in the immunopathogenesis of psoriasis, we have studied the comparative effects of Cyclosporin A (CsA), transforming growth factor-beta (TGF_B), and chloroquine (CQ) on alloantigen presentation by normal human PBM and EC enriched for LC. PBM and EC pretreated with CsA (10 ug/ml x 2 hr at 37°) and then extensively washed failed to stimulate allogeneic T cells in 5 day cultures. 10ng/ml PBM, similarly pretreated with TGF_B (10 ng/ml), also failed to activate allogeneic T cells. However, TGF_B pretreatment had no deleterious effect on APC function of normal LC. CQ (2 ug/ml in culture medium) did not suppress lymphocytes stimulated by allogeneic PBM, but did inhibit T cell responses (50% reduction) to allogeneic EC. These results indicate (a) that the APC functions of LC and PBM are reciprocally resistant and susceptible, respectively, to TGF_B and CQ, and (b) that the APC potential of both PBM and EC are inhibited by CsA. Since the skin lesions of psoriasis are often improved by CsA, but exacerbated by CQ, the contrasting therapeutic effects of these agents may eside in their differential effects on APC present in psoriatic skin. The capacity of LC to resist the inhibitory effects of TGF_B on APC function may offer a clue to a potential physiologic role for this cytokine in dictating appropriate APC function in the epidermal compartment.

DELETION OF AN ULTRA-HIGH-SULFUR PROTEIN FRACTION IN HAIR OF A PATIENT WITH HYPOTRICHOSIS WITH KERATOSIS PILARIS. Satoshi Dekio*, Joji Jidoi*, Takashi Nagashima**, and Yasushi Watanabe***, *Department of Dermatology, Shimane Medical Univ., Izumo, **Department of Dermatology, JR Tokyo General Hosp., Tokyo, and ***Watanabe Dermatology Clinic, Tokyo, Japan.

Hypotrichosis with keratosis pilaris (HTKP) is a rare inborn skin disorder of unknown etiology, which is characterized by sparce, short and brittle hair with keratosis pilaris on the scalp. We recently reported that the fibrous protein composition from the scalp hair of a HTKP patient differed electrophoretically from that of a normal individual. Considering that matrix proteins (MPs) are another important structural constituent of human hair, it is suggested that it is interesting to analyze the hair MP composition of the patient. In the present study, we thus analyzed the composition of the S-carboxymethylated MPs from the scalp hair of the HTKP patient and then compared it with that of the normal individual using tw-dimensional polyacrylamide gel electrophoresis with isoelectric focusing cylindrical gel electrophoresis as the first dimension and sodium dodecyl sulfate slab gel electrophoresis as the second dimension. Consequently, one ultra-high-sulfur protein (UHSP) fraction, which was present on the electrophoregram from the normal individual, was not found anywhere on that from the patient. Since any other differences were not found between the two electrophoregrams, this means that the UHSP fraction of the MPs was deleted. It is thus suggested that the brittleness of the patient's hair might be developed in a close relationship of this deletion of the UHSP.

A 30 KD Peptide Recognized by Anti-Nuclear Matrix Ab Responsible for In Vivo Bound ANA in Direct IF. **JS Deng, M.D., JL Fratto, M Edmond, Ph.D., Departments of Dermatology and Biological Sciences, University of Pittsburgh, PA.** Direct IF of skin from patients with connective tissue diseases reveals immunoreactants at DE junction and/or in vivo bound ANA. The mechanism for such nuclear staining is not fully understood and thought to be related to anti-U1 RNP Ab. The observation of other than anti-U1 RNP Ab in patients with in vivo bound ANA leads us speculate that other factors might be involved. A total of 8 serum samples obtained from different patients with in vivo bound ANA is collected and subjected to the following studies: screening for the presence of auto-antibodies, characterization of specificity of their auto-anti bodies and co-culturing of cells with purified antibodies. 6 of the 8 specimens have high titers of anti-U1 RNP Ab, while one of each for anti-Sm and anti-SSB/La Ab based on double immunodiffusion and immunoprecipitation for RNA. In immunoblot analysis using extract from nuclear matrix, all of them recognize a peptide of 30 KD. Antibody to this 30 KD peptide is purified and isolated from nitrocellulose paper containing 30 KD peptide. This antibody produces a distinct nuclear speckled pattern on HC1-HEp-2 cell slides. This purified antibody is co-cultured with viable HEp-2 cells in vitro for 10 hours. After culture, HEp-2 cells are examined in direct IF, and yield in vivo bound ANA, which is compatible to in vivo ANA pattern in skin direct IF. This study indicates that the antinuclear matrix antibody recognizing 30 KD peptide is responsible for in vivo ANA staining.

BOMBESIN ENHANCES EPIDERMAL REGENERATION IN VIVO. Daniel Deschler, Sigrid Regauer and Carolyn Compton. Department of Pathology, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA.

The effects of insulin-like growth factor I (IGF: 10nM), cholera toxin (CT: 0.1nM), and bombesin (60nM) on re-epithelialization of partial-thickness wounds were studied in 5-month-old Yorkshire pigs. One growth factor per animal was used. Single topical applications were made to eight of sixteen 3x3 cm paravertebral middermal wounds and repeated at 24 hour intervals. Wounds were covered with Tegaderm adhesive dressings. Two growth-factor treated and two untreated control wounds were excised each day for 4 days. The biopsy surface was stained with Toluidine blue and photographed under the microscope. The net area of keratinocyte outgrowth from hair follicles was determined by computerized morphometric analysis in a blinded fashion. Perifollicular outgrowth in sites treated with IGF was not significantly greater compared to untreated control sites until day 3 (increase at day 3 = 26.4%; p=.05). Outgrowth in CT-treated wounds was equal to that of controls at day 1 but was reduced by 22.5% (p=.01) and 20.0% (p=.03) compared to controls on days 2 and 3 respectively. Outgrowth in bombesin-treated sites was also equivalent to controls on day 1, but was increased by 35.1% (p=0.01) on day 2 and by 90.1% (p=.00005) on day 3. These studies demonstrate that topical application of bombesin significantly increases the rate of perifollicular epithelial outgrowth in partial-thickness wounds in vivo and that CT may be inhibitory.

INVOLVEMENT OF PROTEIN KINASE C IN CA^{2+}-MEDIATED DIFFERENTIATION OF CULTURED PRIMARY MOUSE KERATINOCYTES. A.A. Dlugosz, G.R. Pettit*, and S.H. Yuspa, National Cancer Institute, Bethesda, MD, and *Arizona State University, Tempe, AZ.

These studies were undertaken to determine if protein kinase C (PKC) is involved in Ca^{2+}-mediated differentiation of cultured keratinocytes. Primary mouse keratinocytes exposed to the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) for 24 hours are resistant to a second TPA exposure, suggesting that TPA causes down-regulation of PKC in this system as has been reported in several others. 5 day old primary mouse keratinocytes were grown in the presence of 160 nM TPA for 24 hours, washed, then exposed to 0.12 mM Ca^{2+} for 24 hours to induce differentiation. Based on immunoblot analysis, induction of the differentiation-specific keratin 1 (K1) was completely inhibited in TPA pre-treated keratinocytes. Similar results were obtained using phorbol 12,13-dibutyrate, a phorbol ester that is more readily removed from cells after treatment. Bryostatin 1 (BRYO) blocks phorbol ester-mediated responses in several cell types presumably by inactivating PKC. 6 day old primary mouse keratinocyte cultures were induced to differentiate by 0.12 mM Ca^{2+} in the presence or absence of 60 nM BRYO for 48 hours. BRYO inhibited expression of the keratinocyte differentiation markers K1, K10, loricrin, and filaggrin, as assessed by immunoblotting. These data indicate that PKC is necessary for Ca^{2+}-mediated differentiation of cultured primary mouse keratinocytes.

TNF-ALPHA BUT NOT IL 6 INDUCES HLA-DR AND ICAM-1 EXPRESSION IN CULTURED DERMAL MICROVASCULAR ENDOTHELIAL CELLS. ANTI-TNF AB AND $ZNSO_4$ INHIBIT THIS INDUCTION. M. Detmar, S. Tenorio, E. Imcke, Zb. Ruszczak, C.E. Orfanos. Dept. of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Berlin (West), FR Germany.

Dermal microvascular endothelium is a major target for the pro-inflammatory activity of tumor necrosis factor-alpha (TNF). Recently it was suggested that also interleukin 6 (IL 6) may be involved in the mediation of skin inflammation. We investigated the effects of TNF and IL 6 on growth and antigen expression of human dermal microvascular endothelial cells (HDMECs) in vitro.

HDMECs were isolated from the foreskin of neonatals or children by trypsin treatment and continuous Percoll gradient centrifugation and were cultured using EGM (Clonetics). Second passage HDMECs were treated with recombinant TNF (0.1 - 1,000 U/ml) or with recombinant IL 6 (0.1 - 100 U/ml) either alone or in combination with interferon gamma (IFNgamma), with rabbit antibodies (Ab) against TNF and IL 6, with $ZnSO_4$ (0.2mM), with indomethacin or with NDGA for up to 4 days. Growth was assessed by a recently developed fluorometric assay. Expression of HLA-DR and intercellular adhesion molecule ICAM-1 were studied by APAAP immunocytochemistry, FACS analysis, immunogold transmission and scanning electron microscopy.

Neither TNF nor IL 6 markedly influenced the growth of HDMECs, but TNF (1,000 U/ml) induced HLA-DR and, in concentrations from 1 U/ml on, also ICAM-1. The labeling was mainly localized in areas of microvillous cell protrusions at the upper side of the monolayers. In addition, IFNgamma enhanced the TNF mediated ICAM-1 expression, which was suppressed by combination of TNF with anti-TNF Ab, but not with anti-IL 6 Ab. Combination of TNF with $ZnSO_4$ completely inhibited ICAM-1 induction, while indomethacin or NDGA were not effective. IL 6 did not induce expression of HLA-DR or ICAM-1.

In general, IL 6 seems to be of minor importance for the vascular response in skin inflammation, whereas our results indicate a major role of TNF in the mediation of leucocyte adhesion to dermal endothelial cells and in the initiation of inflammatory skin processes.

TREATMENT OF PATIENT SKIN WITH 1 M NaCl INCREASES THE SENSITIVITY OF DIRECT IMMUNOFLUORESCENCE (DIF) MICROSCOPY, UNMASKS IN SITU IMMUNOREACTANTS, AND DISTINGUISHES EPIDERMOLYSIS BULLOSA ACQUISITA (EBA) AND BULLOUS PEMPHIGOID (BP) PATIENTS. N. Domloge-Hultsch, P. Bisalbutra, R. Gammon, and K. Yancey, USUHS, Bethesda, MD and UNC, Chapel Hill, NC.

Prior studies have shown that selected EBA and BP patients can be distinguished by their pattern of basement membrane (BM) autoantibody binding to 1 M NaCl split skin (SSS). To distinguish EBA and BP patients without such circulating autoantibodies, we mapped by DIF the in situ distribution of BM immunoreactants in 22 biopsies from patients with documented BP (N=8), EBA (N=4), or other bullous diseases (N=4) before and after treatment of skin samples with 1 M NaCl for 48 to 72 hrs at 4°C. This treatment produced a cleavage plane between BP antigen and laminin in all samples. All immunoreactants mapped to the base (B) of EBA patients' SSS in contrast to their distribution in BP patients' SSS. Specifically, IgG was found only or predominately in the roof (R) of six BP patients, equally in the R and B of one, and predominately in the B of another. In contrast, C3 was found only or predominately in the B of 5 BP patients' SSS. Since the distribution of IgG rather than that of C3 distinguished EBA and BP patients in these mapping studies, it is of significance that 5 bullous disease patients negative for BM IgG by DIF on untreated skin, showed IgG in situ in their SSS. Moreover, 4 BP patients exhibited C3 or C4d on the surface of basal keratinocytes in their SSS in addition to the BM deposits described above. In summary, these findings have significance for the distinction of EBA and BP patients by DIF, demonstrate that in situ immunoreactants can be unmasked by 1 M NaCl treatment, and show that several patterns of complement deposition are present in BP patients' skin.

A ROLE FOR CYSTEINE AND OTHER SULFHYDRYL COMPOUNDS IN CONTROLLING PIGMENTATION AND PROLIFERATION OF TRANSFORMED MELANOCYTES Walter T. Dixon, James M. Pankovich, Frantisek Alena and Kowichi Jimbow Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Alberta, Canada.

SKMEL 23 human melanoma cells are pigmented cells which synthesize predominantly the reddish brown pigment, pheomelanin, as determined by an HPLC-microassay. Since cysteine/cystine is required during the synthesis of pheomelanin, experiments were carried out to determine the effect of cystine depletion on pigmentation. Cells were grown in cystine-free media and the nature and degree of the resulting melanin production was measured. It was found that SK23 cells "switched" their type of melanogenesis resulting in decreased levels of pheomelanin and correspondingly increased levels of eumelanin. In addition to visible and measurable changes in pigmentation, these cells exhibited increased cell differentiation, morphologically different melanosomes and upregulation of membrane transporters for cysteine/cystine. Since cystine/cysteine is an essential amino acid for in vitro cell growth, the treatment was ultimately cytotoxic. The nature of this cytotoxicity was similar to that previously seen with several potential chemotherapeutic compounds. These observations provide evidence for a common mechanism of action of several melanocytotoxic drugs and may account for their depigmenting or pigment-altering potency. New strategies for melanoma chemotherapy may be possible, based upon direct or indirect manipulation of the levels of sulfhydryl compounds such as cysteine and glutathione.

CYTOCHROME P-450 METABOLISM OF CYCLOSPORINE A BY MICROSOMES ISOLATED FROM PSORIATIC EPIDERMIS, RAT EPIDERMIS AND RAT LIVER. E. Duell, V. Fischer, G. Fisher, A. Astrom, and J. Voorhees, Dept. of Dermatology, Univ. of Michigan, Ann Arbor, MI., USA & Sandoz, Basel, Switz.

Intralesional but not topical cyclosporine A (CsA) is effective in psoriasis (PS). CsA is inactivated by the cytochrome P-450 IIIA (P-450 IIIA) system in liver. This activity in epidermis could inactivate CsA as it penetrates into skin following topical application. P-450 IIIA activity was measured in epidermis and liver.

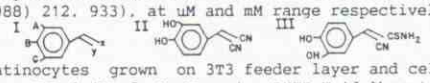
Rats were treated with erythromycin to induce P-450 IIIA activity. NADPH cytochrome c reductase activity was determined as a positive control for presence of microsomal enzymatic activity. In liver from control or treated rats, reductase activity was 20.8±1.3 nmol/min/mg protein (prot). Activity in involved PS, uninvolved PS or rat epidermis was 80% of that in liver (16.9±0.7 nmol/min/mg prot). CsA metabolism (formation of metabolite 1) by liver microsomes of control rats was 3.5±1.2 pmol/min/mg prot (N=3) and was increased to 10.1±3.4 pmol/min/mg prot (N=3) in treated rats. In epidermis insignificant metabolism occurred: 0.3±0.1 pmol/min/mg prot. for control rats (N=3), treated rats (N=3), and uninvolved PS (N=6). CsA metabolism by involved PS was 0.8±0.3 pmol/min/mg prot (N=15), an insignificant 8% of induced liver levels. There was no appreciable metabolism of CsA by keratinocytes in culture. P-450 IIIA mRNA was readily detectable in human liver but not in involved PS or normal epidermis by the polymerase chain reaction. In summary: P-450 IIIA genes and enzymatic activities are expressed in human liver but not in cultured keratinocytes, normal or lesional epidermis.

PLASMA MEMBRANE VISCOSITY MEASUREMENTS ON HUMAN LIVING KERATINOCYTES BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY. William R. Dunham, Cynthia L. Marcelo, Susan B. Klein, Lenore M. Rhodes and Richard H. Sands, Dept. of Dermatology and Biophysics Research Division, The University of Michigan, Ann Arbor, MI.

Rapidly proliferating adult human keratinocytes grown in the absence of polyunsaturated (essential) fatty acids have been postulated by us to have abnormal membrane viscosities. An innovative version of the standard technique of spin-labeling was developed to measure the viscosity of the plasma membrane of living keratinocytes using an electron paramagnetic resonance (epr) spectrometer. This technology uses exacting delivery systems (spin probe [16-doxyl-stearic acid methyl ester] concentrations, carrier concentrations and ratios; incubation temperature and time) and precise epr running conditions (temperature, field modulation amplitude, microwave power, sample and microwave cavity geometry) to measure the viscosity of the plasma membrane of living keratinocyte monolayers. The epr data were analyzed by our newly developed spectral simulation method based on a previously written iterative non-linear minimization algorithm. The output of this algorithm (five parameters) was converted to viscosity by comparing these parameters to an empirically determined library of values. These were obtained by multi-temperature epr experiments on an oil of known viscosities, thus avoiding the usual ambiguities in analyzing the data from spin-label experiments. Reproducible measurements show that the viscosity of keratinocyte plasma membranes can vary five-fold or greater, depending on the cell line, passage number and growth conditions.

TYROSINE KINASE INHIBITORS (TYRPHOSTINS) INHIBIT THE EGF DEPENDENT PROLIFERATION OF NORMAL HUMAN KERATINOCYTES VIA INHIBITION OF EGF RECEPTOR TYROSINE-KINASE - Arie Dvir(*), Alexander Levizki(*), Chaim Gilon(**), Aviv Gazit(**) and Yoram Milner(*), The Dept. of (*) Biological Chemistry and (**) Organic Chemistry, The Hebrew University Jerusalem, Israel.

Tyrphostins are compounds (I) which inhibit the tyrosine kinase (TK) activity of EGF (EGFR) and insulin receptors (Yaish et. al., Science (1988) 212. 933), at uM and mM range respectively.



Human keratinocytes grown on 3T3 feeder layer and cell lines Hacat and carcinoma SCL-1 (Prof. N. Fusenig, DKFZ Heidelberg) were grown in the presence of tyrphostin II and III. II and III inhibited the EGF-dependent proliferation of human cells (IC_{50}II ≈ 15μM, IC_{50}III ≈ 8μM). On the other hand the cell lines' serum induced proliferation was inhibited at higher tyrphostins concentrations (IC_{50}III ≈ 70μM, IC_{50}III ≈ 90μM). TK inhibition (EGFR autophosphorylation and tyrosines in cellular polypeptides) by tyrphostins was checked in digitonin permealized cultures with γ-^{32}P-ATP followed by immuno precipitation (antiEGFR and antityrosine phosphate antibodies). EGF mediated EGFR phosphorylation and TK activities were inhibited (in all cells) in similar Ki's (≈ 4-15μM). It shows that while EGFR activity is the main determinant in mediating normal keratinocytes proliferation some other TK receptor is the determinant in the cell lines growth. The potential use of tyrphostins in EGF dependent hyper-proliferative events in the skin (eg. psoriasis) is evident.

VASOMOTION PROVOKED WHILE SKIN BLOOD FLOW IS INCREASING. Laura Duren and Jonathan Wilkin, Div. of Derm., Ohio State Univ., Columbus, OH.

Oscillatory cutaneous vasomotion may be provoked by stimuli such as postocclusive reactive hyperemia, topical vasodilators, and heat (JID 93:113S. 1989). The vasomotion occurs as the flow returns to baseline from an initial peak. The purpose of this study was to determine if vasomotion could be seen while blood flow was increasing. Heat (up to 45°C) was used to provoke vasomotion in the forearm skin of six subjects. Once rhythmic oscillations had begun, heat was also used to cause several separate increases in flow throughout the duration of the vasomotion. Blood flow was monitored continuously by laser Doppler velocimetry as erythrocyte flux (mV). The results were: 1) vasomotion continued during dilation until the blood flow rose above a threshold unique to each subject, 2) after falling below this threshold, vasomotion resumed. For each subject, threshold values had a standard deviation of less than 50 mV (range=6-48, median=26), 3) if flow did not reach the threshold, vasomotion continued throughout the dilation, and the threshold approximated the blood flow at which vasomotion was first seen in each subject. From these results we conclude that certain characteristics of the microvasculature, yet to be elucidated, determine a threshold above which vasomotion does not occur.

KERATIN FILAMENT MATURATION DURING DIFFERENTIATION OF NORMAL HUMAN EPIDERMIS. Riva Eichner, Diana M. Orentas, and Marc Kahn, Departments of Dermatology and Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD.

In order to investigate in vivo interactions among epidermal keratins, several monoclonal and site-specific anti-keratin antibodies were used in single and double-label indirect immunofluorescence. Specimens of human epidermis and cultured human epidermal cells were observed using both conventional light and confocal microscopy. In normal human epidermis, basal cells contain only keratins K5, K14 and K15, whereas suprabasal cells express additional keratins K1 and K10/11. As previously reported, the majority of antibodies which specifically recognize keratins K5, K14 and K15 in immunoblot analysis, stained only the epidermal basal layer of frozen sections of both neonatal foreskin and adult breast skin, even though these keratins persist in suprabasal cells. A site-specific anti-keratin K14 antibody, however, prepared against a peptide corresponding to a 15 amino acid sequence of a non-helical region in the central rod domain of keratin K14, showed predominantly basal staining, with a gradient of diminished staining in the lower 2-3 spinous layers. These data have implications on the specific interactions between basal cell keratins and differentiation-specific components of suprabasal cells. In addition, these results suggest that although expression of differentiation-specific keratins K1 and K10/11 occurs in the first layer of spinous cells, keratin filament maturation may be a gradual process during the course of epidermal differentiation.

CHARACTERIZATION OF THE RESPONSIVENESS OF HUMAN MELANOCYTES AND MELANOMA CELLS TO CYTOTOXIC LYMPHOCYTES. D Durham-Pierre, RM Halder, CS Walters, HN Pham, CH Evans and E Vanderpool, Depts. of Microbiology, Dermatology and Medicine, Howard University College of Medicine, Wash., DC and NIH, Bethesda, Md.

We have previously shown human neonatal melanocytes (lines 35) and melanoma cells (SK-MEL-28) are susceptible to lymphokine-activated killer (LAK) cells but not to natural killer (NK) cells. The objective of this study was to expand observations to other melanocytes and melanoma lines and assess the effect of leukoregulin (LR), a lymphokine secreted by NK cells on susceptibility of these targets. Additional targets included melanocyte lines 40, 50 and 53 and melanoma lines, SK-MEL-5 and SK-MEL-24. NK and LAK cells were obtained from vitiligo patients and normals. Sensitivity of target cells to effector cells was measured in a 4 hour Cr51 release assay. Results show that when NK cells were boosted with interleukin-2, melanocytes and SK-MEL-28 cells retained their susceptibility ($p < 0.05$). There was a heterogeneity of response of melanocytes to LAK activity; line 40 more susceptible to LAK activity than lines 50 and 53. LR enhanced sensitivity of K562 erythroleukemia cells to NK activity, but response of NK resistant melanocyte and melanoma cells was not increased after LR treatment. LR up-regulated sensitivity of melanocytes and SK-MEL-5 cells to LAK cell cytotoxicity, but LR-treated SK-MEL-28 cells showed no enhanced sensitivity to LAK cells. This data shows that there is heterogeneity in response of human melanocytes and melanoma cells to LAK activity and LR is selective in up-regulating sensitivity to different target cells to LAK cell cytotoxicity.

CYCLOSPORINE SWISH AND SPIT IMPROVES ORAL LICHEN PLANUS IN A DOUBLE-BLIND STUDY. D Eisen, CEM Griffiths, CN Ellis, E Duell, JJ Voorhees, Dept. of Dermatology, Univ. of Michigan, Ann Arbor, MI.

In a double-blind trial, 16 patients with symptomatic oral lichen planus were randomly assigned to receive topical cyclosporine (CSA) 1500 mg/d or its vehicle. Patients swished and expectorated 5 cc of medication t.i.d. After 8 weeks, the 8 CSA recipients exhibited marked improvement in erythema ($p=0.003$), erosion ($p=0.02$), reticulation ($p=0.007$), and symptoms ($p=0.002$); whereas 8 vehicle-treated patients showed no change or minimal improvement. Vehicle-treated patients, after a switch to CSA for 8 weeks, demonstrated improvement similar to that seen in patients who initially received only CSA. There were no systemic side effects. CSA blood levels measured 1-4 hrs after swishing the medication were undetectable in 7 of 16 patients. The remaining patients had levels of 30-151 ng/ml (HPLC). There was no correlation between patients' clinical response and blood levels of CSA. In 6 patients biopsied, epidermal and dermal lymphocyte infiltrates were moderately reduced after CSA therapy as was HLA-DR expression by keratinocytes and lymphocytes; keratinocyte intercellular adhesion molecule-1 (ICAM-1) expression, present in pretreatment specimens, was virtually undetectable following CSA. Lesional buccal mucosa at end of therapy 4 hrs after swishing had 2.1 ng±0.3 CSA/mg tissue wet weight and 17.7 ng±3.9 CSA/mg protein (n=6). In summary: 1)topical CSA is effective for oral lichen planus and thus may be topically effective in other skin diseases; 2)CSA levels in buccal mucosa after topical swish are comparable to those in psoriatic plaques after oral administration of CSA at 14 mg/kg/day; 3)systemic absorption of CSA after topical intraoral application is low.

MURINE FETAL SKIN HARBORS THE PRECURSORS OF DENDRITIC EPIDERMAL T CELLS (DETC). Adelheid Elbe, Elisabeth Payer, Georg Stingl, Div. Cut. Immunobiol., Dept. of Dermatol.I, Univ. of Vienna Med. School, VIRCC at SFI, Vienna, Austria.

Thy-1 antigens and CD3-associated T cell receptors (TCR) are coexpressed on bone marrow-derived dendritic epidermal T cells (DETC) in the adult mouse. We have previously shown that fetal epidermis (day 17 of gestation) contains small numbers of $CD3^-$, $Thy\text{-}1^+$ cells. In order to see whether these cells can qualify as actual precursors of DETC, we transplanted full-thickness grafts from body wall skin of day 17 fetal C57Bl/6 (Thy-1.2) mice onto full-thickness wound beds of B6Pl-Thy-1a (Thy-1.1) animals. At certain timepoints after transplantation, grafts were analyzed for the presence of Thy-1 and CD3 antigens. As opposed to the uniform presence of $Thy\text{-}1.2^+/CD3\text{-}TCR^-$ cells at the time of grafting, examination of the graft on day 11 after transplantation revealed a marked heterogeneity of $Thy\text{-}1.2^+$ epidermal cells including $Thy\text{-}1.2^+/CD3^-$ and $Thy\text{-}1.2^+/CD3^+$ cells of either round or dendritic configuration. 6 weeks after transplantation, essentially all $CD45^+/Thy\text{-}1.2^+$ epidermal cells were anti-CD3ε and anti-TCR Vγ3 (1) reactive, displayed a uniformly dendritic shape and, thus, represent DETC. $Thy\text{-}1.1^+$ cells were only encountered in small numbers on the margins of the graft. Most of these cells were round in shape and $CD3^+$.

Results obtained show that fetal skin contains the precursors of DETC, suggest that these precursors exhibit the $Thy\text{-}1^+/CD3\text{-}TCR^-$ phenotype, and imply that the fetal epidermis can provide stimuli promoting T cell maturation.

1. Havran et al., (1988) Nature, 335: 443-445.

SWEAT GLAND NUMBER AND FUNCTION IN PATCHY AND EXTENSIVE ALOPECIA AREATA. D. Elieff, S. Sundby, *W. Kennedy, and M. Hordinsky, Dept. of Dermatology and *Dept. of Neurology, University of MN Medical School, University of Minnesota, Minneapolis, Minnesota.

Alopecia areata (AA) is a disease characterized by hair cycle and nail abnormalities. In this study we examined another ectodermal derived appendage, the eccrine sweat gland, to determine whether sweat gland number and function are compromised in patients with AA. Sweat gland number and function were evaluated by the silastic imprint method. Sweating was stimulated on the dorsum of the hand (over the first intermetacarpal space) and the foot (over the first intermetatarsal space) by iontophoresis of 1% aqueous pilocarpine nitrate at 2mA for 5 minutes. Following the stimulation period, the test sites were wiped dry, and silastic impression material was immediately applied. After a 4 minute application, the silastic material was peeled from the skin and sweat gland number, mean sweat droplet size, and total area occupied by sweat droplets in the hardened mold was determined using computerized image analysis (International Imaging System). Fifteen females and 3 males between the ages of 3 and 69 years were studied. Seven patients had AA, 4 alopecia totalis (AT), and 7 alopecia universalis (AU). To date, 6 healthy controls matched for age, sex, height, and weight have been studied, and their results compared using Student's t-test analysis to the corresponding 6 patients. All six patients (2 with AA and 4 with AU), showed significantly decreased sweat gland function on the hands and feet as assessed by mean sweat droplet size ($p< .05$). Sweat gland number on the feet of all 6 patients also showed marked decreases ($p<.06$) compared to their controls, however, sweat gland number on the hand was decreased in only 4 of 6 patients studied. Interestingly, the 2 patients that showed little abnormality in hand sweat gland number, also had the least disease extent. These preliminary findings suggest that eccrine sweat gland function may be compromised in patients with AA.

RETINOIC ACID RECEPTOR GENE EXPRESSION IN NORMAL, PSORIATIC, AND RETINOIC ACID-TREATED HUMAN SKIN. JT Elder, GJ Fisher, A Astrom, Q-Y Zhang, A Krust[1], P Chambon[1] and JJ Voorhees, Dept. Derm., Univ. of Michigan, Ann Arbor, MI, and [1]LGME/CNRS and U.184/INSERM, School of Med., Strasbourg, France.

Human skin exhibits a characteristic pleiotypic response to topical retinoic acid, as judged clinically and biochemically. In attempting to understand this response at the molecular level, we have used fast protein liquid chromatography (FPLC), RNA blot hybridization, and the polymerase chain reaction (PCR) to characterize the expression of the retinoic acid receptor (RAR) α, ß, and r genes in adult human epidermis. Keratome biopsies of normal epidermis were homogenized in a hypotonic buffer containing 0.1% digitonin. Extracts were prepared from the resulting nuclei by sonication in 0.6 M NaCl, and incubated with [^{3}H] retinoic acid (RA). Size exclusion FPLC of these extracts revealed 2 peaks of [^{3}H] RA binding at Mr 45 and 18 kDa, in agreement with the expected sizes of RAR and cellular RA binding protein (CRABP). Binding specificity was demonstrated by addition of 100-fold excess unlabeled RA, which reduced [^{3}H] RA binding to near background. Blot hybridization analysis of total RNA extracted from keratome biopsies revealed that RAR-r was the predominant RAR species expressed in vivo, being present at >20-fold higher levels than RAR-α or -ß by comparison with genomic DNA controls. PCR analysis using RAR-r-specific primers detected a prominent PCR product in human epidermal, but not in human liver cDNA, demonstrating tissue specificity of RAR-r expression. Topical treatment with 0.1% RA under occlusion for 4d ($n=14$) failed to reveal autoinduction of RAR mRNA relative to vehicle. Moreover, there was no significant difference in RAR-r mRNA levels in normal and lesional psoriatic epidermis ($n=10$, $p>.05$). RAR-r was the predominant RAR transcript in human KC grown in defined medium (KGM, Clonetics), and its expression was not influenced by treatment with RA (24-48h), phorbol ester (6h), or corticosteroid (4-24h). These results demonstrate high level constitutive expression of RAR-r in human epidermis, and identify RAR-r as the probable molecular target of RA action in human skin.

RATE OF MELANOGENESIS IN CULTURED HUMAN MELANOCYTES IS NOT REGULATED BY TYROSINASE MESSAGE LEVEL. M. Eller, J.M. Naeyaert, P.R. Gordon, and B.A. Gilchrest, USDA Human Nutrition Research Center, Tufts University, Boston MA.

It has been suggested that melanin (mel) production rate varies directly with intracellular mRNA levels of tyrosinase (T), the presumptive rate limiting enzyme in melanogenesis. Using the Pmel34 human T cDNA probe and cultured human melanocytes and human melanoma lines, we correlated TmRNA level with mel/cell as determined by A_{475} and cell counts under basal and stimulated conditions. Among the 10 cell lines, there was a >5-fold difference in TmRNA level by northern blot analysis, but no correlation with mel/cell; amelanotic melanomas indeed had the highest TmRNA levels. In melanocytes treated with 100 uM isobutylmethylxanthine, cell pellets darkened noticably by day 3 and mel/cell doubled by day 7, but TmRNA levels on days 1, 2, 3, 5, and 7 failed to increase compared to untreated controls. In situ hybidization confirmed the presence of TmRNA in all cultured cells, but grain density similarly failed to correlate with mel/cell. These data demonstrate a complete lack of correlation between TmRNA level as measured with the Pmel probe and melanin content and synthesis rate. We conclude that human melanogenesis is regulated either at the mRNA level for a different as yet unrecognized tyrosinase enzyme and/or at the post-transciptional level.

INCREASED EXPRESSION OF LIPOCORTIN I mRNA, BUT NOT LIPOCORTIN I PROTEIN, IN PSORIASIS. JT Elder, RB Pepinsky*, RJ Drugge, JJ Voorhees, and B Wallner*, Department of Dermatology, Univ. of Michigan, Ann Arbor, MI and *Biogen Research Corp., Cambridge, MA

Lipocortin I (lipo I) is a prominent substrate for the epidermal growth factor receptor (EGFR) tyrosine kinase in vitro. Transforming growth factor-α (TGF-α) is the probable endogenous ligand for the EGFR on epidermal keratinocytes and is overexpressed in psoriasis, suggesting that EGFR turnover might be increased in this disease. Since lipo I is likely to be in close proximity to the EGFR on the KC plasma membrane, it is possible that lipo I turnover might also be increased in psoriasis. Therefore, we analyzed levels of lipocortin I mRNA and protein in normal epidermis (NE) and lesional psoriatic epidermis (LPE) in comparison to those of lipocortin II (lipo II). RNA blot hybridization showed that lipocortin I transcripts were markedly overexpressed in many, but not all LPE biopsies, whereas lipocortin II transcripts were unaffected ($_n$=NE, $_p$=LPE; P, 2-tailed t test):

			Lipocortin I		Lipocortin II	
Expt #	$_n$	$_p$	$\bar{x}_p/\bar{x}_n$	P	$\bar{x}_p/\bar{x}_n$	P
1	8	8	6.29	.0006	0.96	.8215
2	10	9	7.56	.0024	1.52	.0037
3	6	8	5.78	.1480	1.09	.5456
4	10	9	5.57	.0002	0.81	.2074

Lipocortin I and II protein levels were measured by Western blotting using monospecific rabbit antisera and detected by the peroxidase technique. Despite the 6-fold increase in lipo I mRNA levels, lipo I protein was not detectably increased in LPE by visual inspection ($n=10$). Lipo II protein levels were also not detectably different in NE and LPE. While differential regulation of lipo I and lipo II translation may explain these results, it is also possible that EGFR and lipo I turnover are coupled and increased in psoriasis, necessitating increased lipo I mRNA levels to maintain constant steady-state levels of lipocortin I protein.

INCREASED EPIDERMAL GROWTH FACTOR RECEPTORS IN MELANOCYTIC LESIONS. Darrel L. Ellis, Lillian B. Nanney, and Lloyd E. King, Jr., Departments of Dermatology, Cell Biology, and Plastic Surgery, Vanderbilt University and Nashville VA Medical Centers, Nashville, TN.

The presence of epidermal growth factor receptors (EGF/R) has been reported in melanomas, and recently was reported to be a marker for progression of malignant potential in melanocytic lesions. To test if the presence of EGF/R indicates malignant potential in melanocytic lesions, we used an EGF/R specific antibody (#451), and a three-step immunoperoxidase procedure to study frozen sections of melanomas, dysplastic nevi, congenital nevi, and normal nevocellular nevi. All melanocytic cell types (nevus cells and melanoma cells) in the lesions studied demonstrated immunoreactive EGF/R staining, although the staining intensity was generally less than the corresponding overlying keratinocyte staining. Increased EGF/R staining was observed in all epidermal keratinocyte layers over the melanocytic lesions. This increased staining decreased to the normal epidermal basal keratinocyte staining pattern in tissue adjacent to the melanocytic lesions. Dermal fibroblasts were uniformly negative for increased EGF/R staining. The presence of EGF/R is therefore not a marker for the progression of malignant potential in melanocytic lesions. However, keratinocyte - melanocytic cell interaction in these melanocytic lesions appears to result in increased immunoreactive EGF/R expression by both cell types.

CONTACT HYPERSENSITIVITY TO THE CHEMICAL CARCINOGENS DIMETHYLBENZ(A)ANTHRACENE AND BENZO(A)PYRENE IN MICE OCCURS INDEPENDENTLY OF THEIR METABOLISM BY THE AH RECEPTOR. CA Elmets and H Mukhtar, Dept of Dermatology, Case Western Reserve University, Cleveland, Ohio.

The initiation of skin cancer in mice by the chemical carcinogens dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BP) requires their binding to the cytoplasmic Ah receptor and metabolism by cytochrome P450 dependent enzymes. Our laboratory has previously shown that DMBA and BP are also potent contact sensitizing agents. In this study, we investigated whether Ah receptor binding and cytochrome P450 dependent enzyme metabolism were required for the development of contact hypersensitivity (CHS). Ah receptor involvement was assessed by pretreating C3H mice with TCDD, which competitively inhibits binding to the Ah receptor, and by attempting immunization in AKR mice, which lack the Ah receptor. In both situations, no alteration in the development of CHS was observed compared to C3H mice. When animals were pretreated with Aroclor 1254, a cytochrome P450 augmenting agent, there was no enhancement of DMBA or BP CHS. Furthermore, in animals that had been immunized with BP, CHS could not be elicited by its cytochrome P450 dependent metabolites. These findings indicate that, in contrast to cutaneous carcinogenesis, Ah receptor binding and cytochrome P450 dependent metabolism are not required for induction of CHS. DMBA and BP-induced skin cancer may therefore be facilitated by a misguided immunological surveillance mechanism that is preferentially directed against the non-carcinogenic parent compound rather than the carcinogenic metabolite.

EFFECT OF 3 AMINOBENZAMIDE (3AB) ON PHOTOCARCINOGENESIS. John H. Epstein, and James E. Cleaver, Departments of Dermatology and Radiobiology, University of California, San Francisco.

The nuclear enzyme poly (ADP-ribose) polymerase covalently modifies many cellular proteins with poly (ADP-ribose) chains using DNA breaks as essential cofactors. Inhibition of this enzyme with 3AB has been shown to both inhibit and enhance radiation and chemically induced transformation of cultured cells. This study examined effects of topical 3AB on UV-carcinogenesis in albino Ucsd strain hairless mice. A hot quartz contact lamp was used as the UVR source. The mice were divided into 3 groups. Gp1 (48) received 102 mj/cm^2 to the posterior 1/2 of the back followed by 0.05 cc 1 molar 3AB in acetone to the posterior and anterior 1/2 of the back 2 times a week. GP2 (49) received UVB and 0.1M 3AB, and GP3 UVB and acetone as in GP1. No significant tumor formation nor irritation occurred on the anterior backskin. The first tumor occurred at 13 weeks in GP1.

	Percent of mice with tumors							
GP\wks	13	17	21	25	29	33	37	41
1	2	4	9	13	21	55	63	81
2	0	0	0	4	19	42	60	73
3	0	0	0	2	4	27	44	67

As per the table, the mice receiving the 1 molar 3AB developed tumors most rapidly and by 29 weeks the effects of 0.1M 3AB became clear. Thus the 3AB enhanced tumorigenesis in a dose response pattern suggesting that inhibition of poly (ADP-ribose) polymerase in vivo may increase transformation effects in vivo.

MULTIPLE PARAMETER ASSESSMENT OF VULVAR IRRITANT DERMATITIS. Peter Elsner, Dorothea Wilhelm, and Howard I. Maibach, Depts. of Dermatology, University of California San Francisco, San Francisco, CA, and University of Wuerzburg, Wuerzburg, FRG.

Very little is known about irritant dermatitis in genital skin. Previous studies comparing the effect of various irritants on forearm and vulvar skin gave conflicting results regarding the relative irritant reactivity of vulvar skin. Since this may be due to the fact that irritant reactions are more difficult to detect visually in vulvar than in forearm skin, there is a need for more sensitive, objective and reproducible methods in the investigation of vulvar irritant dermatitis.

Transepidermal water loss (TEWL), capacitance, pH, blood flow and color reflectance were evaluated for quantifying the irritant response of vulvar and forearm skin to 3% sodium lauryl sulfate in 9 healthy premenopausal women. TEWL, capacitance, pH, blood flow, and all parameters of color reflectance changed significantly in forearm irritant dermatitis. In vulvar irritant dermatitis, however, significant changes were only observed for blood flow and the color reflectance parameters a* and b*. Using the combination of TEWL, capacitance and blood flow, forearm irritant dermatitis was detected with a sensitivity of 84% and a specificity of 100%. The best combination of parameters to detect vulvar irritant dermatitis was pH, blood flow, a* and b*, which had a combined sensitivity of 78% and a specificity of 75%.

It is concluded that TEWL, capacitance, pH, blood flow and color reflectance are suitable techniques to detect and quantify irritant dermatitis in forearm skin. For the study of vulvar irritant dermatitis, however, none of the described techniques is perfect. If objective evaluation of vulvar dermatitis is attempted, however, techniques that measure the inflammatory response such as laser Doppler velocimetry and color reflectance measurement should be preferred.

EFFECT OF TRIGGERING EPIDERMAL Fcγ RECEPTORS ON THE CYTOKINE-INDUCED UPREGULATION OF Ia ANTIGEN ON EPIDERMAL LANGERHANS CELLS. S.P. Epstein, R.L. Baer, G.J. Thorbecke, and D.V. Belsito. Department of Dermatology, New York University Medical Center, New York, NY

Within the epidermis of skin from BALB/c mice, there exists a population of approximately 300 Ia$^-$ Langerhans cells (LC)/mm2 that can be induced to express Ia antigen following incubation in vitro (16 hrs; 37° C) in medium containing any one of a variety of cytokines, including interleukin (IL)-6 (1,000 U/ml). The presence of 5 ug/ml of murine monomeric IgG_{2a} or IgG_3, or 5 ug/ml of heat aggregated IgG_1 or IgG_{2b}, in the medium completely inhibits this cytokine-induced upregulation of Class II antigen without affecting the normal resting level of Ia on LC. The observed inhibition by IgG can be mimicked by Fc fragments of IgG, as well as by PGD_2 (10 uM) and cAMP analogues (0.1-10 uM). PGE_2 over a range of concentrations is not inhibitory. Furthermore, 2'5'-dideoxyadenosine (1 mM), an inhibitor of adenylate cyclase, and indomethacin (10 ug/ml), an inhibitor of cyclooxygenase, completely abrogate the inhibitory effect of each IgG isotype. We conclude that triggering of epidermal Fcγ receptors (R), most likely on LC themselves, results in production of PGD_2 which raises intracellular cAMP. It is this elevation in cAMP which inhibits the cytokine-induced upregulation of Ia antigen. We speculate that FcγR on LC, like those on macrophages, have an important negative feedback role in cell-mediated immune reactions within the skin.

REGULATION OF HUMAN K#3 KERATIN GENE EXPRESSION: IDENTIFICATION OF PROMOTER, ENHANCER AND SILENCER SEQUENCES. H. Epstein, C.-K. Jiang, M. Tomic, I.M. Freedberg, and M. Blumenberg, Department of Dermatology, New York University Medical Center, New York, NY

K#3 is a basic keratin expressed in cornea. To analyze the regulatory sites of its gene, we created constructs in which regions of the K#3 gene control expression of the CAT reporter gene. We have been able to identify, for the first time within a single keratin gene, promoter, enhancer and silencer sequences.

We have demonstrated previously the immediate upstream sequences essential for promoter function and have now characterized a K#3 enhancer, located 1.5 to 2.8 kb upstream from the transcription start. It enhances in stratified epithelial cells, has a weakly positive effect in simple epithelial cells, but is completely inactive in non-epithelial cells. It functions in either orientation, both upstream and downstream from the transcription start, and it is active with heterologous promoters.

Since it enhances in all stratified epithelial cells, the sequence does not contain cornea-specific regulatory sites. These are provided by a tissue-specific silencer, located in the transcribed region of the gene. This silencer functions in either orientation with various promoters. Since it silences in all cell types tested except cornea, it is cornea-specific.

Regulation of K#3 keratin gene expression thus depends on three distinct regulatory DNA segments: the proximal upstream promoter, an enhancer in the distal upstream segment, and a silencer within the transcribed region of the gene.

FIBRONECTIN, LAMININ, TYPE IV COLLAGEN MODULATION OF CULTURED HUMAN MELANOMA CELL MIGRATION RATE: TIME LAPSE VIDEO IMAGE ANALYSIS STUDY. Takafumi Etoh, H. Randolph Byers, *Arthur J. Sober and Martin C. Mihm Jr. Dermatopathology Division and *Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts.

Recent studies on cell migration in culture of human primary, recurrent cutaneous and metastatic melanoma using time-lapse image analysis suggested that primary cutaneous melanoma exhibited less migratory behavior than metastatic melanoma cells (Byers et al., J. Invest. Derm. 92:409a,1989). In order to evaluate the effects of various extracellular matrix substrates on the migration of melanoma cells from different stages of tumor progression, one primary, one recurrent cutaneous and two metastatic cell lines were studied using laminin (LN), fibronectin (FN) and type IV collagen coated substrates. The dose dependency of melanoma migration rate was examined relative to substrate coating concentrations of 0, 0.1, 1.0, 10, 100 μg/ml of each extracellular matrix protein.

FN increased the migration rate of one metastatic pleomorphic cell line; however, it had no effect on another metastatic spindle cell line. Likewise, the recurrent cutaneous melanoma cell line also exhibited an increased migration rate with FN. The primary melanoma cell line did not show an increase of the migration rate with FN. In contrast, LN increased the migration rate of both metastatic melanoma cell lines; however, the pleomorphic melanoma cell line exhibited the increase at a lower substrate concentration than the spindle cell line. LN had no effect on the migration of the recurrent cutaneous or primary melanoma cell lines. Type IV collagen promoted migration in all cell lines tested.

The differential effects of these extracellular matrix proteins on melanoma cell lines of different stages of tumor progression indicates complexity in the control of human melanoma cell migration. These studies may lead to a better understanding of the behavior of primary melanoma compared to its metastasized counterpart.

TWENTY PERCENT OF BIOPSIES FROM SUNEXPOSED SKIN OF NORMAL YOUNG ADULTS DEMONSTRATE POSITIVE IMMUNOFLOURESCENCE. Vilma C. Fabre, Steven J. Hodge and Jeffrey P. Callen, Division of Dermatology, Department of Medicine, Univ. of Louisville, Louisville, Ky.

Direct cutaneous immunoflourescence (IF) indicative of lupus erythematosus (LE) is characterized by the presence of IgM, IgG or both with or without C3 in a continous band of granular IF along the dermal-epidermal junction (DEJ). The detection of this particular pattern of IF in biopsies obtained from lesional skin has been used in differentiating lesions of cutaneous LE from other dermatoses. When a biopsy is obtained from clinically normal skin, the finding of positive IF supports the diagnosis of SLE and may be indicative of decreased long-term survival . The prevalence of positive IF varies between the different types of cutaneous LE and depends upon whether the biopsy is obtained from lesional vs. nonlesional skin or sunexposed vs. non-sunexposed skin. We performed a study designed to determine the rate of false-positive IF in sunexposed skin of normal young adults. We recruited 50 normal healthy adults, ages 18 - 40, who did not have a history of any systemic diseases, dermatoses, photosensity eruption, and were not on medications. Paired 3mm punch biopsy specimens were obtained from each volunteer from the lateral neck (below the angle of the jaw) and the non-sunexposed buttocks. The biopsies were examined without knowledge of patient or location using polyvalent anti-human IgG, IgM and IgA. All paired biopsies found to be positive on initial screening were reexamined using fracionated monospecific immunoglobulin against IgG, IgM, IgA and C3 . The data from the positive biopsies are shown in the table below and are characterized according to immunoglobulin class.

Site of Biopsy	Polyvalent IgG,M&A	IgG	IgM	IgA	C3
Exposed	10	9	5	8	0
Non-exposed	0	0	0	0	0

We observed a bright continous band of granular IF along the DEJ in 10 of 50 specimens obtained from the sunexposed neck but none from the non-sunexposed buttocks.There was significant variability according to immunoglobulin class. The false-positive rate of IF in sunexposed skin in our group is 20%. This information suggests that in the evaluation of a patient suspected of having lesions of cutaneous LE, a positive result by immunoflourescence obtained from sunexposed skin is nonspecific and therefore adds little information to the clinical and histopathologic findings.

Altered G-protein Associated with Adenylate Cyclase Abnormality in Monocytes from Patients with Atopic Dermatitis. Linda Fancher, Rita Lloyd, Jeff Kosokoff, Sai Chan and Jon Hanifin. Dept. of Dermatology, Oregon Health Sciences University, Portland, OR.

We have reported increased GTP-stimulated adenylate cyclase(AC) activity in monocytes from patients with atopic dermatitis(AD) correlates with increased binding of GTP to G-proteins. Increased cAMP production may relate to either diminished effect of the inhibitory subunit(Gi), or to increased stimulatory subunit(Gs) coupling. We probed with Cholera toxin(CT) and Pertussis toxin(PT) and report here abnormal AD monocyte G-protein ADP-ribosylation.

Monocyte membranes were homogenized and spun at 45,000g, then resuspended by sonication. AC was assayed by Salomon's method using ^{32}P-ATP as substrate. ^{32}P-NAD was used as label for ADP-ribosylation of G-proteins in the presence of 100 µg/ml of CT or 10 µg/ml of PT. After SDS-PAGE and autoradiography, ADP-ribosylation was measured by densitometry.

CT and PT caused dose-dependent ADP-ribosylation of G-proteins from monocyte membranes. AD monocyte 41Kdalton Gi was ADP-ribosylated at a greatly reduced level in the presence of PT. PT stimulated normal monocyte AC activity but failed to stimulate AC in AD monocytes. The 43Kdalton Gs from AD monocytes was more than two-fold higher than normal Gs, a difference also reflected in CT stimulated AC activity.

These results correspond with the concept that βγ subunit dissociation of Gi is a negative feedback inhibitor for Gs. Thus, abnormal Gi in AD might lead to excessive AC activity, requiring a compensatory increase in cAMP-phosphodiesterase activity to maintain cellular homeostasis.

TERBINAFINE LEVELS IN SERUM, STRATUM CORNEUM, DERMIS-EPIDERMIS (WITHOUT STRATUM CORNEUM), HAIR, SEBUM AND ECCRINE SWEAT DURING AND AFTER 250 MG TERBINAFINE ORALLY ONCE PER DAY IN MAN.

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We determined terbinafine levels in serum, stratum corneum, dermis-epidermis (without stratum corneum), hair, sebum and eccrine sweat before, during and after 250 mg doses orally to volunteers once daily. Terbinafine is concentrated rapidly in stratum corneum (up to 9.1 µg/g of tissue) primarily by diffusion from the vascular system through the dermis-epidermis. It also reaches high concentration in sebum (up to 45.1 µg/ml) after several days and continue to concentrate in sebum for up to two days after discontinuation of drug. Hair concentration reach levels of 2.6 µg/g of tissue indicating high drug levels in and around the hair follicle. It is not found in sweat. Plasma levels range between 0.1 and 1.0 µg/ml. There is an increase in accumulation of drug in stratum corneum of tenfold by day 2 and 70 fold by day 12. Elimination of drug from tissue occurs with a half-life of 4 to 5 days and with the potential for drug levels above fungicidal concentrations for dermatophytes for more than 3 weeks. The tissue pharmacokinetic profile of terbinafine is similar to that of another lipophilic drug, itraconazole, but is very different from ketoconazole and griseofulvin. Higher levels of terbinafine are achieved than of either of the imidazoles and remain longer than griseofulvin.

CHROMOSOMAL MAPPING OF THE HUMAN ELASTIN GENE TO LOCUS 7q11.2. Michael Fazio, Marie-Geneviéve Mattei, Mon-Li Chu, Donald Black, Ellen Solomon, Jeffrey Davidson, and Jouni Uitto. Thomas Jefferson University, Philadelphia, PA; Inserm U242 Marseille, France; ICRF, London, UK; and Vanderbilt University, Nashville, TN.

We have recently isolated cDNAs corresponding to the entire encoded primary sequence of human elastin (J. Invest. Dermatol. 91:458-464, 1988). In this study, elastin cDNAs were utilized in molecular hybridizations to establish the location of the human elastin gene. First, in situ hybridizations were performed using metaphase chromosomes from PHA-stimulated human peripheral blood leukocytes. Examination of 100 metaphase cells revealed a total of 189 silver grains associated with the total human genome, and 71 grains (37.5%) localized on chromosome 7. The distribution of grains was concentrated on the long arm of chromosome 7 within [q11.1-21.1] region, and the peak number of grains coincided with the locus 7q11.2. Secondly, hybridizations with a panel of human-rodent cell hybrids, using either a human cDNA or sheep genomic probe, showed a concordance with chromosome 7. The latter hybridizations also excluded chromosome 2. These results contradict a previous publication that suggested, on the basis of hybridizations with a 0.4 kb cDNA corresponding to the 3' untranslated sequences of human elastin mRNA, that the elastin gene resides on chromosome 2. Our results indicate that human elastin gene is located in the proximal region of the long arm of chromosome 7. The precise localization of the elastin gene in human genome is useful in establishing genetic linkage between inheritance of an elastin allele and a heritable cutaneous disorder.

IDENTIFICATION OF A HUMAN DESMOSOMAL CALMODULIN BINDING PROTEIN. J.A. Fairley, G.A. Scott, K. Jensen, L.A.Goldsmith, L.A. Diaz, Depts of Dermatology, Medical College of Wisconsin, Milwaukee WI and Univ of Rochester, Rochester NY.

Calcium can regulate epidermal proliferation and differentiation in vitro. Since many of the effects of calcium are modulated by its binding protein calmodulin (CaM) we have begun to analyze epidermal calmodulin binding proteins. Human epidermis was extracted using 50 mM Tris. The CaM binding proteins were partially purified by CaM affinity chromatography and released with EGTA, concentrated and used to generate murine monoclonal antibodies. The specificity of the monoclonal antibodies against CaM binding proteins was tested by immunofluorescence (IF), immunoblotting (IB) and immunoelectronmicroscopy (IEM). By IF one monoclonal bound strongly to the ICS of cryosections of normal human epidermis and diseased epidermis in pemphigus vulgaris (n:3) and bullous pemphigoid (n:1) but failed to bind involved epidermis in pemphigus foliaceus (n:4). Viable human keratinocytes in culture did not stain however, the cells became reactive after treatment with 0.1% saponin suggesting an intracellular epitope. IEM mapped the protein to the desmosome. IB analysis identified a protein of 165 kD and pI 5.4 from a total human epidermal extract. Sequential extraction of epidermis showed a large fraction of the antigen bound to the insoluble cell envelope pool, shown by EM to contain desmosomal remnants. This data indicates the presence of a desmosomal CaM-binding protein in human epidermis of similar MW and pI as desmoglein 1. This protein may be involved in calcium-dependent assembly of desmosomes.

ISOLATION OF COMPLEMENTARY DNA FOR DESMOPLAKIN I BY ANTIBODY SCREENING OF A LAMBDA GT11 LIBRARY. Steven R. Feldman and Edward J. O'Keefe, Department of Dermatology, University of North Carolina, Chapel Hill, North Carolina.

Desmoplakins I and II are high molecular weight components of desmosomes with molecular weights of approximately 250-285,000 dalton and 215-225,000 dalton, respectively. Much of desmoplakin I is homologous to desmoplakin II, although desmoplakin I also contains a unique region recognized by some antibodies. A complementary DNA (cDNA) clone of desmoplakin I was obtained by screening a random primed cDNA library prepared from a human epidermoid carcinoma cell line, and the identity of this clone was confirmed by epitope selection. RNA was obtained from the A388 human epidermoid carcinoma cell line by guanidine thiocyanate extraction, and the messenger RNA (mRNA) component was isolated by double oligo-dT affinity chromatography. cDNA prepared by random priming on this mRNA template was size selected to exclude low molecular weight inserts and was cloned into a lambda gt11 vector. Screening of 60,000 recombinants of the library with affinity purified anti-desmoplakin I antibody resulted in identification of four clones. Plaque purification resulted in isolation of one clone. The cDNA insert size of this clone, 1500 base pairs, was determined by gel electrophoresis of the product of the polymerase chain reaction using oligonucleotides which bind to the lambda gt11 arms flanking the insert site. The identity of this clone was confirmed by demonstrating that antibody affinity purified on the protein product of this clone bound desmoplakins I and II in a Western blot. Antibody from the same sera affinity purified on the protein products of unselected clones of the library did not bind desmoplakins. The isolated clone appears to represent cDNA for the common region of desmoplakins I and II.

PILOT STUDY TO EVALUATE THE EFFECT OF TOPICAL BETAMETHASONE DIPROPIONATE ON THE PERCUTANEOUS ABSORPTION OF MINOXIDIL FROM A 5% TOPICAL SOLUTION. James J. Ferry and Virginia C. Fiedler, Clinical Pharmacokinetics and Dermatology Depts., The Upjohn Co., Kalamazoo, MI.

Eleven healthy volunteers completed this two way crossover study to determine the effect of betamethasone dipropionate (BD) on percutaneous minoxidil (M) absorption. Subjects were dosed twice daily for five days with one ml of a 5% topical M solution in each of two treatments: within 30 minutes of either a 1/8 gm dose of BD cream 0.05%, or a vehicle control cream. All doses were applied to a 100 cm^2 area of the volar surface of the forearm. Suction-induced subepidermal blisters were formed 12 hours after the last applied doses and aspirated to assess local tissue concentrations of M. Twenty-four hour urine samples were collected on study day 5 to assess systemic M absorption. The mean (±SD) M blister fluid concentration of 42.5 (±20.4) mcg/ml was 1.7-fold greater for dosing with BD compared with vehicle control cream ($p<0.05$). In contrast, steady-state urinary excretion of M tended to be greater for dosing with vehicle control cream, 313 (±194) vs 221 (±94.1) mcg. The results suggest that BD increases local tissue M concentrations and decreases systemic M absorption, presumably as a result of reduced M clearance secondary to local vasoconstriction induced by BD. Augmentation of local tissue M concentrations may explain, in part, an apparent synergism observed in preliminary studies of the combination for treatment of alopecia areata.

TOPICAL RETINOIC ACID INCREASES TRANSFORMING GROWTH FACTOR-β1 IMMUNOREACTIVITY BUT NOT ITS mRNA IN HUMAN EPIDERMIS IN VIVO. GJ Fisher, JT Elder, D Eisen, H Higley, S Chu, L Ellingsworth, JJ Voorhees, Dept. Dermatology, Univ. Mich., Ann Arbor, MI and Celtrix Laboratoriess, Collagen Corp., Palo Alto, CA.

The molecular mechanisms through which retinoic acid (RA) exerts its effects on skin are largely unknown. Transforming growth factors-β (TGF-β) are a family of proteins that modulate growth and differentiation of many cell types including keratinocytes (KC). In mouse epidermis, topical RA specifically induces TGF-β2 protein and mRNA (Glick et al. Cell Reg. 1:87, 1989), suggesting that it may mediate some of the effects of RA. We have treated human subjects with 0.1% RA cream (Retin-A^R) for four days under occlusion and measured TGF-β1 and TGF-β2 immunoreactive protein and mRNA. Paraffin sections from RA and vehicle-treated skin (N=8) were fixed in Bouin's solution and stained with anti-peptide rabbit antibodies specific for TGF-β1 or TGF-β2, using the immunoperoxidase method. Sections were also stained with collodial iron for glycosaminoglycans (GAG). Total RNA was extracted from keratome biopsies, and analyzed by northern hybridization using specific cDNA probes for TGF-β1 or TGF-β2. Vehicle-treated specimens showed no TGF-β1 immunoreativity, while in RA-treated specimens TGF-β1 staining was pronounced. Staining was most prominent in intercellular spaces between KCs in the upper two thirds of the epidermis. TGF-β2 in vehicle-treated samples displayed minimal matrix-associated staining near hair follicles and slight intracellular KC reactivity. No changes in staining intensity or distribution of TGF-β2 were observed after RA treatment. RA markedly increased collodial iron positive, hyaluronidase sensitive material (i.e. GAG), which was distributed coincidentally with TGF-β1 immunoreactivity. TGF-β1 mRNA was detected in vehicle and RA-treated samples. In contrast to immunoreactivity, however, RA did not elevate TGF-β1 mRNA. TGF-β2 mRNA was not detectable in vehicle or RA-treated samples. These data demonstrate that in human epidermis RA induces TGF-β1, but not TGF-β2 as reported in mouse epidermis. This appears to occur through a post transcriptional mechanism, which does not require increased mRNA levels.

Immunohistochemical Localization of Transforming Growth Factor-Alpha Expression In Human Skin. **Eric Finzi and Thomas Horn, Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland.**

Transforming growth factor (TGF-∝) is a 50-amino acid polypeptide in its fully processed form that is structurally related to epidermal growth factor (EGF) and binds to the EGF receptor. TGF-∝ was originally shown to be synthesized by neoplastic cells but has since been reported to be synthesized by normal human keratinocytes and overexpressed in psoriatic epidermis. Data will be presented showing immunohistochemical localization of TGF-∝ expression in seborrheic keratoses, keratoacanthomas, squamous cell carcinomas, prurigo nodules, psoriasis, and other skin diseases.

CELLULAR, IMMUNOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF TOPICAL RETINOIC ACID-TREATED HUMAN SKIN. GJ Fisher, J Esmann, CEM Griffiths, HS Talwar, EA Duell, C Hammerberg, JT Elder, GD Karabin, BJ Nickoloff, KD Cooper, CJ Petersen, JJ Voorhees, Dept. Dermatology & Pathology, Univ. Michigan, Ann Arbor, MI.

Topical retinoic acid (RA) results in histological and clinical improvement of sun-exposed skin. Daily RA application typically produces within 2-5 days an erythematous scaling reaction, which lessens with continued usage. The cellular, immunological and biochemical basis of the retinoid reaction and its role in the repair of photodamaged skin are not known. To investigate the retinoid reaction, we have treated non sun-exposed skin with 0.1% RA cream (Retin-A^R) under occlusion for 4 days and examined changes in: 1) histology, 2) expression of cell surface molecules, 3) enzymes and second messengers of the phospholipase C (PLC)/protein kinase C (PKC) signal transduction system, and 4) levels of eicosanoids and interleukin-1 (IL-1). Compared to vehicle, RA caused significant epidermal cell growth as judged by increased epidermal thickness (RA, 0.092±0.012μm; CTRL, 0.054±0.005μm; N=9; $p<0.001$) and mitotic figures (RA, 3.8±3.9/10HPF; CTRL, 0.11±0.33/10HPF; N=9; $p<0.004$). Increased numbers of CD4+ T-cells accompanied by prominence of dermal dendrocytes, and focal keratinocyte expression of intercellular adhesion molecule-1 (ICAM-1) were observed in RA-treated biopsies. In cultured human keratinocytes, RA (1-10μg/ml) treatment for 24 hours elevated ICAM-1 mRNA, as determined by northern hybridization, suggesting direct modulation of ICAM-1 expression by RA. RA also caused increased phosphoinositide-specific PLC activity (2.5-fold; $p<0.001$) and diacylglycerol content (1.6-fold; $p<0.001$). PKC activity was reduced by one third ($p<0.001$) in both the soluble and membrane fractions. No changes were observed in levels of eicosanoids or IL-1α, however, IL-1β protein and mRNA were significantly decreased. These results demonstrate that treatment of skin with RA induces an acute erythematous response, which includes epidermal hyperplasia, a mild perivascular T-cell infiltrate, keratinocyte ICAM-1 expression and modulation of PLC/PKC-mediated signal transduction.

EPIDERMAL GROWTH FACTOR STIMULATES PHOSPHOLIPASE C AND PHOSPHOLIPASE D ACTIVITIES IN HUMAN DERMAL FIBROBLASTS. GJ Fisher, JJ Baldassare, PA Henderson, JJ Voorhees, Dept. of Dermatology, Univ. of Michigan, Ann Arbor, MI and American Red Cross, St Louis, MO.

1,2-diacylglycerol (DAG), which is formed as a result of agonist-stimulated phospholipase-catalyzed phospholipid hydrolysis, is the physiological activator of protein kinase C (PK-C). Both phorbol esters, which directly activate PK-C, and epidermal growth factor (EGF) stimulate growth of human dermal fibroblasts (HDF). We therefore investigated whether EGF induces DAG formation in these cells. HDF were treated with EGF (50ng/ml) for various times and DAG mass measured by enzymatic assay. EGF elevated DAG (2-fold) within 1 minute. Maximal accumulation (4-fold) ocurred at 5 minutes. DAG may be formed from phosphatidylcholine (PC), phosphatidylethanolamine (PE), or phosphatidylinositol (PI) by the direct action of phospholipase C (PLC) and/or the combined actions of phospholipase D (PLD) plus phosphatidic acid (PA) phosphatase (PAPase). To determine the precursors and enzymatic pathways for EGF-induced DAG formation, cellular PC, PE and PI were labelled with [^{3}H]choline, [^{3}H]ethanolamine, or [^{3}H]inositol, respectively, and the radioactive products formed in response to EGF measured. In cells labelled with [^{3}H]choline, PC hydrolysis products were increased 2-fold within 5 minutes of EGF addition. No hydrolysis of PE or PI was observed. Quantitation of the PC metabolites by TLC revealed equivalent elevations in choline phosphate and choline, the products of PLC and PLD-catalyzed PC hydrolysis, respectively. To assess the relative contributions of these two enzymes to DAG formation, HDF were treated with EGF in the presence of [^{14}C]ethanol (0.5%). Under these conditions, PLD catalyzes exchange of choline from PC with ethanol to form phosphatidylethanol (P-ETOH), instead of PA, which cannot be broken down by PAPase to DAG. P-ETOH was increased nearly 5-fold, 5 minutes after addition of EGF. This did not, however, effect EGF-induced DAG accumulation. Thus, although EGF activates both PLC and PLD in HDF, the pathway for DAG formation appears to be via PLC. The accumulation of DAG provides a molecular link between EGF and PK-C.

DIFFERENTIAL EXPRESSION OF PROTEIN KINASE C ISOZYMES IN PSORIATIC LESIONS VERSUS NORMAL EPIDERMIS. GJ Fisher, A Tavakkol, CEM Griffiths, O Baadsgaard, KD Cooper, JJ Voorhees, Dept. Dermatology, Univ. Michigan, Ann Arbor, MI

Protein kinase C (PKC) mediates many of the responses of cells to extracellular signals, such as hormones, growth factors and inflammatory mediators. PKC is a family of several closely related isozymes, which display subtle differences in regulation and function. Total PKC activity is reduced by one half, in psoriatic compared to normal epidermis. The impact of this on PKC isozyme expression is not known. We have investigated: 1) the composition, 2) mRNA levels, and 3) cellular localization of PKC isozymes in normal and psoriatic epidermis. Resolution of PKC isozymes by hydroxyapitite chromatography revealed the presence of PKCα and PKCβ, but not PKC ., in both normal and psoriatic epidermis. In normal epidermis the ratio of the activities of PKCα to PKCβ was 1:2. In psoriatic epidermis, PKCβ was preferentially decreased, such that the ratio of the activities of PKCα to PKCβ was reversed to 2:1. The identity of the resolved isozymes and their relative abundances were confirmed by immunoblot with isozyme specific monoclonal antibodies (MoAB). Hybridization analysis of poly-A^+ RNA with PKC isozyme-specific cDNA probes, revealed transcripts for PKCα and PKCβ, but not PKC ., in both normal and psoriatic epidermis. No differences between normal and psoriatic epidermis in mRNA band intensities for PKCα or PKCβ were observed. Thus, the reduction of PKC in psoriatic lesions is not due to a deficiency in steady state mRNA. Frozen sections from normal and psoriatic skin were stained using immunoperoxidase with MoAB's specific for PKCα and PKCβ. In normal skin, staining of PKCα was confined to keratinocytes (KC) in the lower epidermis, while PKCβ was localized to KC in the mid-epidermis, with intense staining of some cells. Double immunofluorescence staining revealed that Leu 6 positive Langerhans cells (LC) accounted for the intense PKCβ staining. In psoriatic epidermis, staining of both PKCα and PKCβ in KC and LC was greatly diminished. Differential expression of PKCα and PKCβ within the epidermis may permit transduction of functionally distinct signals. In psoriasis, decreased PKC, with preferential loss of PKCβ, may result in altered responses of KC and LC to extracellular signals and LC.

THY-1 EXPRESSION AND T CELL RECEPTOR TYPE IN MYCOSIS FUNGOIDES AND BENIGN DERMATOSES. David P. Fivenson, Lawrence Rheins, James J. Nordlund, Edward A. Krull. Departments of Dermatology, Henry Ford Hospital, Detroit, Michigan and University of Cincinnati, Cincinnati, Ohio.

We have studied human Thy-1 expression in mycosis fungoides (MF) and benign dermatoses. Correlations between Thy-1 and T cell receptor (TCR) type are made. 18 cases of MF (5 patch, 8 plaque, 6 tumor stage, 1 Woringer-Kolopp disease), 8 normal skin, 8 nummular dermatitis, 8 lupus erythematosus, 1 lichen planus, and 2 parapsoriasis were evaluated. Immunoperoxidase studies using the ABC technique on serial frozen sections were performed. Primary antibodies were antihuman Thy-1, anti TCR α, anti TCR β, and anti TCR δ. Thy-1 was expressed in all MF cases with 10 - 100% of dermal lymphocytes positive. Epidermotropic cells did not express Thy-1. Thy-1 was seen perivascularly and on fibroblasts in normals and patients as previously reported. Normals and benign dermatoses differed from MF by the lack of Thy-1 on dermal lymphocytes. TCR α/β predominated in all cases, with TCR γ/δ expression noted on < 5% dermal lymphocytes per 400x field. Epidermotropic cells in MF expressed 75% TCR α/β and 25% TCR γ/δ. Thy-1+ dermal lymphocytes were consistently TCR α/β + and TCR γ/δ - on serial sections. The function of the increased Thy-1 expression on the dermal but not epidermotropic lymphocytes in MF is unclear. The increased percentage of TCR γ/δ +, T cells in the epidermis may induce immune tolerance to the transformed lymphocytes of MF.

LOW DOSE CYCLOSPORINE IMPROVES PSORIASIS: A DOUBLE-BLIND STUDY. MS Fradin, MD Brown, CN Ellis, T Annesley, TH Hamilton, E Duell, KD Cooper, JJ Voorhees, Dept. of Dermatology, Univ. of Michigan, Ann Arbor, MI.

In a sixteen week double-blind trial, 85 patients with recalcitrant psoriasis were randomly assigned to receive either 3.0, 5.0, 7.5 mg/kg/d cyclosporine A (CsA) or its vehicle. After 8 weeks, the percentage of patients rated as either clear or almost clear in each group were: vehicle (0%), 3 mg/kg/d (35%), 5 mg/kg/d (61%), and 7.5 mg/kg/d (80%)($p=.0001$). After week 8, dosage increments were allowed for patients who failed to achieve at least moderate improvement, and by week 16, 78% of all patients were clear or almost clear. Lesion levels of CsA (ng/mg protein) were found to correlate with both CsA trough blood levels ($r=.74$, $p<.0001$) and oral dose of CsA ($r=.67$, $p<.0001$). To investigate the effect of CsA on glomerular filtration rates, a subgroup of 43 randomly chosen patients were evaluated with iothalamate clearances. After 8 weeks, the mean decline in clearance from all groups, compared to baseline, was 13% ($p=.0003$). Decrease in clearance was not significantly correlated with dosage ($p=.17$) but was correlated with CsA trough blood levels ($p=.002$). Skin anergy panel testing to investigate the effect of CsA on delayed type hypersensitivity revealed a 40% decline in the number of positive responses at week 16 ($p=.003$). Statistically significant laboratory side effects included: increases in serum creatinine, BUN, bilirubin and cholesterol and decreases in serum magnesium. We conclude that low dose CsA:1)is effective in the treatment of psoriasis; 2)causes a moderate decline in cell mediated immunity; 3)causes statistically significant changes in renal function and other laboratory parameters, the clinical significance of which is unclear.

ANTISENSE PROFILAGGRIN mRNA ALTERS EPIDERMAL DIFFERENTIATION IN VITRO. P. Fleckman, P.V.Haydock, S. Brumbaugh, B.A.Dale and K.A.Holbrook, Univ of Washington, Seattle, WA.

Profilaggrin (proFG) expression in a cultured rat epidermal cell line was disrupted by a plasmid that directs synthesis of antisense proFG RNA. The hypothesis to be tested was that the phenotype of ichthyosis vulgaris, where proFG is decreased or absent, could be mimicked. A rat epidermal cell line (a gift of H Baden and J Kubilus) was transfected by the calcium phosphate technique. Nontransfected and G418 resistant cells not containing antisense plasmid served as controls. ProFG and K1 are normally detected only in confluent cells. Antisense plasmid was detected in both subconfluent and confluent transfectants by Northern blot analysis of keratinocyte RNA using strand specific probes generated from a pGEM subclone containing a portion of rat proFG cDNA. In contrast, proFG mRNA was detectable only in confluent cells, and was diminished in transfected cells. Control cells showed an abrubt decrease in actin and G3PDH mRNA at confluence that was delayed in transfectants. Immunoblot and immunofluorescence analysis showed that proFG and filaggrin protein expression was delayed and decreased in transfected cells. EM showed that cornified cellular envelope formation was delayed and keratohyaline granules were decreased, globular and heterogeneous in transfectants cells. Surprisingly, the precipitous drop in S-35 methionine incorporation into cytoskeletal protein seen at confluence was delayed in transfected cells. Unlike ichthyosis vulgaris keratinocytes where keratin expression is unaffected, K1 protein synthesis and expression was decreased in transfected cells. We conclude that, rather than producing the ichthyosis vulgaris phenotype, antisense proFG has a global effect on in vitro differentiation of rat epidermal keratinocytes.

PERCUTANEOUS ABSORPTION OF FLUOCINONIDE IN MAN: ASSESSMENT OF RELATIVE BIOEQUIVALENCE OF 0.05% LIDEX OINTMENT AND SOLUTION. Thomas J. Franz and Paul A. Lehman, Division of Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Fluocinonide, the 21-acetate ester of fluocinolone acetonide, is a widely used high potency corticosteroid. The objective of this study was to determine if different formulations of the same strength (0.05% Lidex ointment (O) and 0.05% Lidex solution (S)) demonstrate equivalent bioavailability, as assessed by measurement of Fluocinonide percutaneous absorption.

Three healthy adult volunteers received applications of both vehicles to normal intact forearm skin (100 mg/40 cm^2) while six additional volunteers received applications of both vehicles to scotch tape stripped skin (to simulate pathologically damaged skin). Penetration was determined by monitoring tritiated Fluocinonide distribution and excretion in blood, urine and feces over 5 days following a 10 hour topical exposure. At 10 hours, the surface was washed to remove remaining drug.

The percutaneous absorption of Fluocinonide from the two vehicles was not significantly different when compared on normal or taped stripped skin. As expected though, tape stripped skin was more permeable than normal intact skin. Urinary excretion of Lidex when applied to normal intact skin was 0.29% (O) and 0.39% (S) of the applied dose whereas from stripped skin penetration was 2.66% (O) and 3.09% (S). Peak urinary excretion occurred 10 hours after the topical application. An additional 6% of the dose from both vehicles was measured in the fecal excretion. Unexpectedly, the surface wash recovered more drug from the surface of the tape stripped skin than from the surface of normal intact skin.

DERMAL COLLAGEN FIBRILS ARE HYBRIDS OF TYPE I AND TYPE III COLLAGEN MOLECULES. Raul Fleischmajer, Douglas MacDonald, Jerome S. Perlish, Robert E. Burgeson, and Jeffrey Kopp, Mount Sinai School of Medicine, New York, Shriners Hospital for Crippled Children, Portland, OR, National Institutes of Health, Bethesda, MD.

It has been suggested that collagen fibrils with 60 nm periodicity consist of hybrids of type I and type III collagens. This is based on the assumption that all fibrils are coated with type III collagen although conclusive evidence of hybridization is missing. To clarify this problem adult dermis was treated with 8 M urea for 24 hours, thus, disrupting collagen fibrils into microfibrils. This was followed by single and double indirect immunoelectron microscopy with 5 and 10 nm gold particles and purified antibodies against the C-telopeptide domain of type I collagen (polyclonal IgG, rabbit origin) and against the helical domain of type III collagen (monoclonal, IgM, mouse origin). Intact collagen fibrils (not disrupted) showed type III collagen at the periphery in a spiral conformation but no labeling with type I collagen antibodies, suggesting that the epitopes for type I collagen were masked. Collagen fibrils disrupted into bundles of microfibrils revealed type I collagen throughout the fibril except for the periphery where type III collagen was present. Almost no type III collagen was noted in the interior of the collagen fibrils. This study demonstrates that dermal fibrillar collagen consists of hybrids of type I-type III collagen molecules. Since type III collagen is only present at the periphery it suggests that this collagen plays a role in the regulation of fibril diameter.

THE USE OF WATER PERMEABILITY AS A MEANS OF VALIDATION FOR SKIN INTEGRITY IN IN-VITRO PERCUTANEOUS ABSORPTION STUDIES. Thomas J. Franz and Paul A. Lehman Division of Comparative Toxicology, National Center for Toxicological Research, Jefferson, Arkansas.

Since the advent of in-vitro skin permeation models for the assessment of percutaneous absorption of pharmaceuticals and toxic chemicals a particular concern has been the suitability of the skin sample being used. Having used water permeability in our laboratory as a test to assess skin integrity for normal barrier function prior to its use in diffusion studies we can now report representative normal values and criteria for acceptability of a given skin sample.

The water permeability test is conducted on skin sections mounted onto Franz diffusion chambers with saline as the receiver solution. Sufficient tritiated water is applied to totally cover the skin surface (100 ul/cm^2) for a five minute exposure duration whereupon the water is quickly removed. The receiver solution is sampled one hour after the water application and its tritium content quantified.

Full thickness skin shows a water penetration of 0.197 ± 0.015 ul (n=31 donors), dermatomed skin a value of 0.383 ± 0.061 ul (n=11), and epidermal preparations a value of 0.363 ± 0.027 ul (n=23).

Skin sections which are physically damaged clearly show a water penetration >5 ul. Skin sections which have demonstrated a water penetration value greater than 200% of the normal value have been found to also demonstrate increased permeability to the primary compound subsequently tested indicating abnormal or atypical barrier function. The use of this test for skin integrity will insure that the skin sections used will accurately represent normal intact barrier function from a given donor and will provide a means of standardized uniformity of results for pharmaceutical kinetic studies or toxin risk assessment when studied by in vitro skin permeation models.

CHARACTERIZATION OF THE TRANSCRIPTION SPECIFICITY OF EPIDERMAL KERATIN GENE PROMOTERS. I.M. Freedberg, C.-K. Jiang, M. Tomic, H. Epstein, and M. Blumenberg, Department of Dermatology, New York University Medical Center, New York, N.Y.

We have cloned the promoters of the epidermal keratin genes K#5, K#6, K#10 and K#14. Constructs containing promoter sequences driving a reporter gene (CAT) were introduced into various mammalian cell cultures. None of the promoters were active in non-epithelial cells, indicating that each is epithelial-specific. Promoters for the basal cell-specific keratins, K#5 and K#14, were active in HeLa (simple epithelium) as well as other epithelial cells. Since K#5 and K#14 are not expressed in simple epithelia *in vivo*, these two promoter constructs contain sites for epithelial-specific expression, but not for down-regulation in simple epithelium.

The K#6 promoter was active only in primary cultures of cornea and esophagus, which express endogenous K#6, but not in HeLa or in fibroblasts. The K#6 construct, thus, has all the information for directing high levels of transcription in hyperproliferating cells of stratified epithelial origin.

The K#10 promoter was active only at a very low level in primary cultures of stratification-competent cells of non-epidermal origin. This suggests more stringent requirements for expression than we found for the K#5, K#6 and K#14 promoters. For high level expression, K#10 may require epidermal keratinocyte-specific factors, and/or the cells may have to be in the process of differentiation.

Our data indicate that the promoter regions of these keratin genes contain sites which specifically regulate keratin synthesis.

DIHYDROLIPOATE INHIBITS REACTIVE OXYGEN SPECIES MEDIATED SKIN INFLAMMATION. J. Fuchs, R. Milbradt, and G. Zimmer, Zentrum der Dermatologie und Venerologie, and Zentrum der Biologischen Chemie, Universitatklinikum, Frankfort, FRG.

Reactive oxygen species play an important role in skin inflammation by mediating tissue injury. We induced skin inflammation in mice by ultraviolet B, intradermal injection of xanthine-oxidase/hypoxanthine, ferrous/hydrogen peroxide, t-butylhydroperoxide, and adriamycin. The antiinflammatory activity of the antioxidant dihydrolipoate was analyzed in these systems. Intensity of dermatitis was quantitated by measuring skin thickness with a spring loaded caliper gauge, and scoring erythema, hemorrhage and ulceration according to a simple rating. Intradermal injection of dihydrolipoate immediately after administration of the oxidant generating system, significantly inhibited the inflammatory reactions caused by ultraviolet B, xanthine-oxidase/hypoxanthine, and t-butylhydroperoxide. Adriamycin caused skin inflammation and necrosis was only slightly inhibited, and ferrous/hydrogen peroxide induced inflammation was augmented. The study indicates that dihydrolipoate inhibits skin inflammation in mice mediated by different generators of reactive oxygen species. The rather proinflammatory effect of dihydrolipoate in presence of ferrous/hydrogen peroxide is explained by metal ion catalyzed thiol autoxidation and formation of sulfur and oxygen centered free radicals. It is tempting to suggest that dihydrolipoate may be useful clinically as an antiinflammatory agent in dermatitis with pathophysiologically significant involvement of reactive oxygen species.

WATER PERMEATION OF REAGGREGATED STRATUM CORNEUM WITH MODEL LIPIDS. Stig E. Friberg, Ibrahim Kayali, Wayne Beckerman, Linda D. Rhein, and Anthony Simion, Department of Chemistry, Clarkson University, Potsdam, NY and Colgate Palmolive Research Center, Piscataway, NJ.

Corneocytes were separated from stratum corneum by extraction of the lipids. The corneocytes were aggregated with model lipids to form a membrane. The transport of water through the membrane was found to be similar to earlier published values for reaggregated stratum corneum with the indigenous lipids. The results confirmed that model lipids in which the free fatty acids have been partially saponified to give a layered structure may be used for the recombination of stratum corneum according to Smith, et al. Further experiments showed that the complete lipid spectrum of the model was not necessary. Results using the saponified free fatty acids as lipids gave water penetration values not significantly different from the results using the complete model or the indigenous lipids. These findings lend support to our earlier suggestion that the barrier to water transport through stratum corneum rests with the layered structure per se and not with the chemical structure of the individual components.

THE CADHERINS : CELL - CELL ADHESION MOLECULES POSSIBLY CONTROL PATHOGENESIS OF PEMPHIGUS Fukumi Furukawa, Kimio Fujii, Norihisa Matsuyoshi, Yuji Horiguchi and Sadao Imamura Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, 606 Japan

Pemphigus is histologically characterized by the loss of coherence between the epidermal cells (acantholysis), in which the role of plasminogen activator and antibody dependence has been hotly debated. Cadherins are a family of glycoproteins involved in the Ca^{2+}-dependent cell-cell adhesion mechanism and protease-sensitive. Since cadherins are cell-cell adhesion molecules, it is of interest to determine whether or not cadherins are involved in the pathogenesis of pemphigus.

Skin specimens were obtained from 10 normal volunteers and 6 patients of pemphigus vulgaris. Keratinocytes from neonatal foreskins were cultured in MCDB 153 medium. Monoclonal anti-epithelial (E) and anti-placental (P) cadherin antibodies were kind gifts of Prof. Takeichi. Indirect immunofluorescent (IF) study, FACS analysis and immunoblotting study were performed.

In normal skin tissue, P-cadherin was detected in epidermal basal cells and E-cadherin was detected in whole epidermal cells. E-cadherin showed the intercellular distribution. Interestingly, acantholytic cells and the epidermal cells near blister in pemphigus lacked the reactivity to E-cadherin. In the cell culture, high Ca^{2+} (0.3~0.6 mM) produced much higher IF intensity with intercellular distribution of anti-E or P cadherin antibody than low Ca^{2+} (0.1 mM), whereas cells in 0.03 mM Ca^{2+} showed no reactivity. These results were confirmed by FACS analysis. The reactivity was not blocked by sera of pemphigus patients. High Ca^{2+} increased the amount of P-cadherin in cultured keratinocytes.

These results suggest that cell-cell adhesion molecules play a significant role in the loss of coherence between the epidermal cells.

ELECTRON PARAMAGNETIC RESONANCE TOMOGRAPHY IN SKIN. J. Fuchs, N. Groth, and T. Herrling. Zentrum der Dermatologie und Venerologie, Universitätsklinikum, Frankfurt, FRG, and Zentrum für wissenschaftlichen Gerätebau, Akademie der Wissenschaften der DDR, Berlin, GDR.

Electron paramagnetic resonance (EPR) spectroscopy is the most direct approach to determining the existence, role and importance of paramagnetic species (e.g., free radicals) in biological material. In particular, EPR tomography allows spatial resolution of paramagnetic centers in different tissue planes. We have developed an EPR tomography method using conventional X-band EPR spectroscopy combined with the modulated gradient technique. This approach allows direct measurement of the spatial resolution of free radicals in tissues and the possibility for obtaining an EPR spectrum in a selected volume part. The objective of this study was to analyze spatial distribution of free radicals in skin of hairless mice. Nitroxide type persistent free radicals were chosen as model compounds. We measured liberation and permeation kinetics of charged and uncharged nitroxide spin probes, reduction kinetics of nitroxides having different redox potentials, and employed covalent spin probes to measure thiol group reactivity in different skin layers. Evaluation of the results show that the EPR tomography modality has unique advantages over other methods. Free radical processes as well as pharmacokinetics of spin probes and spin labeled drugs may be monitored non invasively in skin. The technique also allows spatial resolution of biochemical reactions in skin, e.g., mapping thiol reactivity and one electron transfer reactions of persistent free radical species.

THE EFFECT OF CYTOCHALASIN D AND COLCHICINE ON ORGAN CULTURED EPIDERMIS WITH FOGO SELVAGEM IGG. S Futamura, S Kitada, Y Takagi, Y Asada. Dept. of Dermatology, Kansai Medical Univ., Osaka, Japan.

Pemphigus is an autoimmune blistering disease, as a result of cell detachment termed acantholysis. The relationship between desmosome and tonofilament has been discussed in connection with the pathomechanism of pemphigus. However there is no report in which the role of actin filament (AF) and tublin (TB) on the disassembly of desmosomes in pemphigus-Fogo Selvagem (FS) was studied.

The subject of the present study is to investigate the effect of cytochalasin D (CD:this binds to AF and injures) and colchicine (COL:this binds to tublin and injures) on FS IgG-induced acantholysis in mice by organ culture system. Explants were treated with CD or COL prior to culture in media containing FS-IgG ; parallel untreated control culture was also prepared. Then skin was studied at 0, 1, 3, 6, 12, 15, 18, 24, 30 hours(hs) by immunofluorescence (IF), electron microscopy (EM) and immuno-EM. The distribution of AF and TB in keratinocytes was studied by IF to make sure the effect of CD and COL. CD treatment caused the redistribution of AF into numerous aggregates . FS-IgG could be seen in ICS by IF and on the surface of the keratinocytes by Immuno-EM in all series of organ explant. By EM, localized acantholysis was detected at 21hs in the specimen treated with or without COL.

However, in the specimen treated with CD, acantholysis was not seen until 30 hs. Namely CD inhibited acantholysis by FS-IgG, while COL did not inhibit it. These results indicate that AF have a important role on the disassembly of desmosomes induced by FS-IgG.

MODULATION OF GM-CSF AND IL-3 PRODUCTION BY A MURINE KERATINOCYTE CELL LINE FOLLOWING ULTRAVIOLET RADIATION EXPOSURE. R.L. Gallo, D.N. Sauder*, T.L. Knisely, and R.D. Granstein, Department of Dermatology, Harvard Medical School, Boston, MA., and *Division of Dermatology, McMaster University, Hamilton, Ontario

Regulation of IL-3 and Granulocyte/Macrophage Colony-Stimulating Factor (GM-CSF) production was investigated in the transformed murine keratinocyte (KC) cell line PAM 212. Release of cytokines was compared between rapidly dividing subconfluent cultures and growth inhibited confluent cultures. Cells were exposed to ultraviolet radiation (UVR) from FS-40 fluorescent tubes (67% emission between 280-320nm), and cytokine activity in culture supernatants assayed by proliferation of the cell lines FDC-P1 or DA-1. Neutralization experiments with antibodies directed against IL-3 or GM-CSF revealed a significant increase in the release of GM-CSF 5 hrs following 240 J/m^2 UVR exposure of subconfluent cells. Conversely, confluent cell cultures responded to UVR with an apparent decrease in both GM-CSF and IL-3 activity. Slot-blot analysis of mRNA in subconfluent PAM 212 cultures revealed a transient increase in mRNA for both cytokines 1 hr following exposure to 600 J/m^2 of UVR, but no change in mRNA levels when cells were grown to confluence prior to irradiation. Inhibition of mRNA synthesis with 20 μM cordycepin blocked the UVR induced increase in release of GM-CSF from subconfluent cultures. Removal of extracellular calcium prior to and during irradiation of subconfluent KC similarly inhibited an increase in secreted bioactivity, while augmenting calcium permeability with ionomycin stimulated release from both control and UVR exposed cells. These results suggest differential regulation of GM-CSF and IL-3 release from KC. Factors important to regulation of these growth factors include state of cell growth and calcium concentration.

IMMUNOHISTOCHEMICAL LOCALIZATION OF MELANOMA-ASSOCIATED ANTIGENS AND HORMONE RECEPTORS IN A CONGENITAL MELANOMA CELL LINE. S. Garnis-Jones, S. Carriere, M. Feeley, P. Huang and M. Herlyn, University of Ottawa, Canada, and the Wistar Institute, Philadelphia, USA.

Melanoma-associated antigen and hormone receptor expression in a congenital melanoma cell line developed at the Children's Hospital of Eastern Ontario, was compared to that in MEWO-LC1, an established adult melanoma cell line.

The cells were harvested, centrifuged, counted and cytocentrifuged when growth had reached a plateau. Fixation was in dehydrated acetone. Monoclonal antibodies to oncofetal proteins, disialogangliosides, basement membrane proteins, HLA-DR, p97, placental antigen, fibroblast antigen, other carbohydrate antigens similar to disialogangliosides, and receptors to ME 20.4s, 425s, 452 developed at the Wistar Institute, as well as commercially available monoclonal antibodies to progesterone and testosterone(Medix Biotech, Calif.), were then used with the avidin biotin complex method in the localization of the antigens and receptors.

The congenital melanoma cell line expressed oncofetal proteins, the tissue receptor to 20.4s, 452, 425s, the placental antigen, disialogangliosides and several carbohydrate antigens similar to disialogangliosides which the MEWO-LC1 did not express. Neither cell line expressed basement membrane protein, HLA-DR, the fibroblast antigen and p97. In terms of the hormone receptors, the receptor for testosterone was present in both while progesterone was absent.

These findings confirm the heterogeneity of antigenic expression in cell culture.

IMMUNOPHENOTYPIC AND FUNCTIONAL COMPARISON OF HDMEC DERIVED FROM NEONATAL AND ADULT SKIN. E. Garcia-Gonzalez, M. Baucom, Y. Xu, R. Swerlick, T. Lawley, Emory Univ., Atlanta, GA.

Human dermal microvascular endothelial cells (HDMEC) differ from large vessel human umbilical vein endothelial cells (HUVE) in growth requirements, tube forming ability, mediator secretion and expression of cell surface antigens. Thus, it is important to utilize HDMEC and not HUVE in studies of cutaneous inflammation, wound healing, and immune responses. However, almost all studies utilize HDMEC cells isolated and purified from neonatal foreskins and not from adult skin. We have performed immunophenotypic and functional comparative studies using neonatal and adult HDMEC. Neonatal and adult HDMEC were isolated by identical procedures, cultured under the same conditions and pure cultures of both were obtained. In studies of tube forming capacity neonatal and adult HDMEC were plated on matrigel and showed morphological differentiation at 8 h. Flow cytometric analysis showed that both expressed class I, LFA-3, ICAM-1, CD9, CD44, leukocyte response integrin, and the vitronectin receptor in similar amounts. ICAM-1, but not other cell adhesion molecules, could be upregulated in both types of MEC by pretreatment with IL-1 and IFN- . Functional studies using a T cell adherence radioassay showed similar T cell binding to neonatal (37.6 + 8%) and adult (29 + 13%) HDMEC. IL-1 (40 u/ml for 4 h) caused upregulation of T cell binding to both in neonatal 38 + 8% to 55 + 17% vs. adult 29 + 13% to 61 + 26%. Monoclonal antibody to LFA-1 caused inhibition of T cell binding to both (neonatal 49% vs. adult 55%). This study shows for the first time that neonatal and adult HDMEC possess similar immunophenotypic and functional properties and that neonatal HDMEC are thus an excellent model for the study of endothelial cell function in human skin.

REDUCED NATURAL KILLER CELL ACTIVATION AND DIMINISHED INDUCTION OF INTERFERON-γ ACTIVITY IN LEUKOCYTES FROM PATIENTS WITH XERODERMA PIGMENTOSUM. A. A. Gaspari, T. A. Fleisher, and K. H. Kraemer, Dermatology Branch, NCI, Clin. Pathology Dept. CC, and Lab. of Molecular Carcinogenesis, NCI, NIH, Bethesda, MD.

Xeroderma pigmentosum (XP) is a rare disorder with photosensitivity, increased skin cancer susceptibility, and defective DNA repair. In order to confirm a previous report of a possible natural killer (NK) cell immune defect in XP, we examined NK cell function in peripheral blood leukocytes of 8 XP patients who had multiple skin cancers. While the numbers of T-cell subsets were normal, NK cell function (lysis of ^{51}Cr labelled K562 and MOLT 4 tumor cells) was 6-50% of the normal controls in 5 of the XP patients and 100% of controls in the other 3. Leukocytes from 4 XP patients (2 with normal and 2 with low NK activity) were assayed for their response to poly IC, a biological response modifier that enhances NK cell function. The 4 XP patients had a 1% increase in NK cell function; controls had a 46% increase. Because poly IC is also a potent interferon inducer, we examined interferon-γ (IFN-γ) production in response to poly IC, or other interferon inducers such as tumor cells or interleukin-2 (IL-2). Leukocytes from XP patients produced substantially less IFN-γ activity (poly IC: 0-31% of normal control activity; tumor cells: 0-32%, IL-2: 15-63%). This data indicates that XP patients are heterogeneous in their NK cell function. In contrast, leukocytes from all XP patients tested showed reduced augmentation of NK activity and diminished induction of IFN-γ activity in response to *in vitro* stimulation. These immunological abnormalities may be related to the high frequency of skin cancers in these XP patients.

EARLY GENETIC EVENTS FOLLOWING ULTRAVIOLET IRRADIATION OF HUMAN KERATINOCYTES. M. Garmyn, M. Yaar, N. Holbrook, B.A. Gilchrest, USDA Human Nutritian Research Center, Tufts University, Boston, MA; and NIA Gerontology Research Center, Baltimore, MD

In order to explore the early genetic events indicative of UV injury and repair we exposed near confluent newborn keratinocytes to 64 mj/cm2 of solar simulated light (SSL, metered at 295 nm) using an appropriately filtered Kratos LH 153 Solar Simulator. Total cellular RNA was harvested at 0.5,1,2,4,8,14 and 24 hours after irradiation for northern blotting. Specific mRNA was detected using cDNA probes to heat shock protein 70 (HSP 70) and ubiquitin genes known to be involved in the cellular injury, and to a recently cloned growth arrest and DNA damage inducible gene *gadd* 153 isolated by hybridization subtraction from chinese hamster ovary cells after 254-nm UV irradiation. Ubiquitin and HSP70 were abundantly expressed in sham irradiated control samples and no further induction could be detected in SSL irradiated cells. In contrast, at 8 hours there was a 20 fold increase in mRNA level for *gadd* 153 in SSL irradiated versus sham irradiated keratinocytes. *gadd* 153 thus appears to be a sensitive marker of solar simulated UV damage and/or repair.To our knowledge, *gadd* 153 is the first gene demonstrated to be induced by SSL in cultured keratinocytes.

PHOTOACTIVATABLE ANTISENSE DNA: UVA PHOTOACTIVATION ENHANCES THE EFFECTS OF ANTISENSE DNA. F. Gasparro, M. O'Malley, L. Amici, and R. Edelson, Department of Dermatology, Yale U., New Haven, CT.

To determine the optimal concentrations for the effect of antisense alone and photoactivatable antisense DNA plus UVA on the sensitization of normally resistant cells to ampicillin, E. coli has been used as a model system. A strain transformed with a 2.96kb phagemid (pBLUESCRIPT) is resistant to the effects of ampicillin. Using a 9 base oligonucleotide (9mer) complementary to a region of the β-lactamase gene which includes the initiating methionine codon, cells were rendered sensitive to ampicillin to a level of 18.7%±2.5% of controls at 40 uM 9mer. Lower concentrations of 10 uM and 20 uM had significantly reduced effects: 90.4%±1.7% and 82.7%±2.1%, respectively. When the 9mer is modified at one of its thymine residues by the incorporation of the 4',5'-monoadduct of 8-methoxypsoralen (9merMA), cells can be sensitized to the effects of ampicillin with only 0.4 uM in combination with 1 J/cm^2 UVA (31.2%±8.5%). UVA alone or 9merMA alone displayed only slight non-specific effects (>85%). The activity of the gene for β-galactosidase, which is also located in the same phagemid, was examined using a colorimetric assay. Treatment with 0.4 uM 9merMA and 1 J/cm^2 UVA did not significantly reduce the activity of this enzyme below control levels (90.8%±1.2%). Thus, the incorporation of the psoralen monoadduct (which is the precursor to the psoralen crosslink) within the antisense strand enhanced the activity of this new form of photobiological control of genomic activity.

LIPID PEROXIDATION STIMULATES COLLAGEN SYNTHESIS IN HUMAN DERMAL FIBROBLASTS. Jeffrey C. Geesin, Laura J. Hendricks, Joel S. Gordon, and Richard A. Berg, Department of Biochemistry, Rutgers University, and the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, New Jersey, and Johnson and Johnson Consumer Products, Skillman, New Jersey.

Ascorbate (50-200 μM) has been shown to stimulate collagen synthesis in cultured dermal fibroblasts by increasing transcription of the collagen genes. Since ascorbate causes lipid peroxidation in an iron dependent reaction, we tested inhibitors of lipid peroxidation on ascorbate-stimulated collagen synthesis. Traditional lipid peroxidation inhibitors, such as propyl gallate, cobalt chloride, and α-naphthol, inhibit ascorbate-stimulated collagen synthesis and lipid peroxidation at similar concentrations for both effects. Mannitol, a water soluble anti-oxidant, has no effect on ascorbate-stimulated collagen synthesis and lipid peroxidation, while α-tocopherol and natural retinoids inhibit both, indicating that lipophilic anti-oxidants can prevent these responses. Superoxide dismutase, catalase, and their polyethylene glycol conjugates did not inhibit the ascorbate stimulated collagen systhesis. These results suggest a correlation between collagen synthesis and lipid peroxidation and suggest an explanation for the fibrosis occurring in oxidant induced tissue injury.

TISSUE-SPECIFICITY AND MODULATION BY RETINOIC ACID OF THE PROMOTER ACTIVITY OF THE HUMAN PAPILLOMAVIRUS TYPE-18 LONG CONTROL REGION (HPV18-LCR). Catherine Gerst, Catherine Bailly, Michel Darmon, and Bruno A. Bernard, Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, France.

Papillomaviruses are small epitheliotropic DNA viruses that replicate as extrachromosomal plasmids in infected cells. To explore to which extent the strict epitheliotropism of human papillomaviruses could be attributed to the promoter activity of the viral long control region (LCR), we transfected into various cell types the construct pH18CAT containing the CAT reporter gene under the control of the HPV18-LCR. We show that the promoter activity of the HPV18-LCR is active only in keratinocytes, and we suggest that the epitheliotropism of the virus is mainly mediated by the LCR. To further characterize this tissue-specificity, DNAse footprinting experiments were performed to possibly identify the DNA sequences which are specifically recognized by keratinocyte nuclear proteins. Moreover, we had previously shown that addition of retinoic acid (RA) to human keratinocytes grown in 10% delipidized serum resulted into the activation of transcription from the construct pH18CAT. In order to investigate whether addition of this potent modulator of keratinocyte differentiation would result into modifications of the subset of factors able to interact with the HPV18-LCR, we performed DNAse footprinting experiments with nuclear extracts of keratinocytes grown in the presence or the absence of RA.

PEMPHIGUS FOLIACEUS IgG1 AND IgG4 BINDING TO CULTURED HUMAN KERATINOCYTES. William Geoghegan and Robert Jordon, Dept of Dermatology, Univ of TX Medical School, Houston, TX.

Pemphigus foliaceus (PF) IgG1 and IgG4 were shown to exhibit two different binding patterns on cultured human keratinocytes (HuK) (Kim et al, J Lab Clin Med 1990). The purpose of this investigation was to determine if both PF IgG subclasses bound to the same or to different HuK and to compare the binding patterns of the two subclasses to the binding pattern exhibited by anti-desmoglein; desmoglein is reportedly bound by some PF sera. HuK were cultured in MCDB 153 in 0.1 mM calcium (Ca) and switched to 1.2 mM Ca 24 hours before use. IgG subclass binding was detected by a) HP6025, anti-human IgG4, or by HP6069, anti-human IgG1, followed by fluorescein goat anti-mouse IgG or b) in HP6069 followed by fluorescein goat anti-mouse IgG and biotinylated HP 6025 followed by Texas Red streptavidin. PF sera exhibited binding of IgG1 and IgG4 in course granular and speckeled patterns respectively. IgG4 binding was restricted mostly to large flat HuK; occasionally binding was observed on smaller HuK. IgG1 binding was generally restricted to smaller HuK. IgG1 and IgG4 binding was almost always restricted to HuK in the upper cell layers. Preliminary results suggest that some HuK bind both IgG4 and IgG1. Anti-desmoglein bound mostly to cell contact areas. The patterns exhibited by PF IgG1 and by PF IgG4 were unlike those of anti-desmoglein; they generally covered the entire cell. These data suggest that the PF IgG1 and IgG4 binding patterns represent antibodies to non-desmoglein antigens and that these antigens are not necessarily expressed simultaneously.

ABNORMAL INTERCELLULAR LAMELLAE AND DESMOSOMES IN CONGENITAL ICHTHYOSIFORM ERYTHRODERMA. Ruby G. Ghadially, Eddie S.Y. Hou, G.K. Menon, Noel Taylor, Mary L. Williams, Peter M. Elias. Dermatology Serv., VAMC, and Dept. of Dermatology, UCSF, San Francisco, CA.

Lipids in mammalian stratum corneum (SC) are segregated to intercellular domains where they appear to regulate barrier function and desquamation. CIE is an autosomal recessive form of ichthyosis characterized by alterations in triglyceride, fatty acid, and n-alkane scale content. To delineate whether altered lipids lead to abnormalities in SC intercellular domains, biopsies from CIE patients (n=3) were compared with normals and patients with other DOC. Aldehyde pre-fixed specimens were post-fixed in 0.2% ruthenium tetroxide or 1% osmium tetroxide with 0.5% $K_4Fe(CN)_6$ for 0.5 hrs, and processed for electron microscopy. Parallel specimens were processed for cytochemical demonstration of acid and neutral lipases, using Tween 85 as the substrate. Whereas normal intercellular bilayers consist of one or more basic units, composed of electron-dense lamellae alternating with continuous and interrupted electron-lucent lamellae, in CIE the central, electron-dense band and electron-lucent bands of the basic unit are diminished leading to a reduction in the width of the basic unit. In addition, the number of desmosomes is increased within the outer layers of the SC in CIE, indicative of impaired degradation, apparently due to impaired delivery of hydrolases, as shown by the cytochemical demonstration of a paucity of lipases in the intercellular domains of CIE. These studies suggest that the abnormal desquamation in CIE may be due to abnormal intercellular lamellar structure and/or desmosomal persistence.

CELLULAR CHANGES IN DRAINING LYMPH NODES FOLLOWING TOPICAL APPLICATION OF A PHOTOALLERGEN. G. Frank Gerberick, Cindy A. Ryan, E. Robert Fletcher, Angela D. Howard, and Michael K. Robinson, The Procter & Gamble Co, Miami Valley Laboratories, Cincinnati, Ohio.

Current evidence suggests that during the induction phase of a contact sensitization response, dendritic epidermal Langerhans cells bind to sensitizers in the epidermis, migrate via the afferent lymphatics to regional draining lymph nodes (DLN), and initiate antigen-specific T-lymphocyte proliferation. Draining lymph node cells isolated from mice 48 hours after topical exposure to 1% TCSA plus 10 J/cm^2 UVA (TCSA+UVA) demonstrated a 2 to 5 fold increase in the number of dendritic cells (DC) as compared with control mice treated with vehicle + UVA radiation. Peak DC accumulation occurred at 48 hrs post application and at a TCSA dose of 1%. Photospecificity was evident in that mice irradiated prior to treatment with TCSA (UVA/TCSA) demonstrated no significant increase in DC accumulation. DC accumulation in the DLN of TCSA+UVA treated mice was followed by a significant increase in total lymph node cellularity which continued to increase up to 4 days following test material application. DC number and lymph node cellularity changes were also observed in mice treated with the contact sensitizer, oxazolone, but were not observed in mice treated with the phototoxin, 8-methoxypsoralen (8-MOP)+UVA, or with the irritant, sodium lauryl sulfate (SLS). An in vivo ^{3}H-TdR incorporation assay was used to assess the proliferative activity of cells isolated from the draining lymph nodes of photosensitized mice. A 20-fold increase in ^{3}H-TdR incorporation was observed in mice treated with TCSA+UVA as compared with naive, vehicle, or UVA/TCSA treated mice. Moreover, mice treated with oxazolone, but not with SLS or 8-MOP+UVA, demonstrated a significant increase in incorporation of ^{3}H-TdR. Dendritic cells isolated from mice treated with TCSA+UVA, but not those from naive mice or mice treated with UVA/TCSA, were capable of stimulating in vitro proliferation by responder lymphocytes from mice photosensitized to TCSA. Lymphocytes from untreated mice or mice photosensitized with musk ambrette demonstrated a much lower response to DC isolated from TCSA+UVA treated mice, demonstrating the specificity of the reaction. Moreover, DC-depleted lymph node cells were unable to stimulate in vitro proliferation in responder lymphocytes. These results suggest that application of TCSA+UVA induces cellular and functional changes in the lymph node DC consistent with their involvement in the induction phase of a contact photoallergic reaction.

PASSIVE TRANSFER OF SERUM FROM PATIENTS WITH ALOPECIA AREATA TO NUDE MICE GRAFTED WITH HUMAN SCALP SKIN. Amos Gilhar, Michael David, Thomas Pillar, and Bedia Assy, Skin Research Laboratory, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Previously we have demonstrated regrowth of hair in scalp skin grafts taken from patients with alopecia areata (AA) and alopecia universalis (AU) following engraftment onto nude mice. The aim of the present study was to use our murine model to determine whether serum of patients with AA and AU plays a role in the process of hair loss in AA/AU. Seven patients were included in this study and 13 biopsies were obtained from each patient for grafting. Forty-five mice were grafted with transplants obtained from patients. A group of grafted mice were given patients' serum and another group normal serum. The mice were treated topically with cyclosporine (CyA) or olive oil. Hair growth was noted in most grafts. Intravenous injections of serum did not prevent or inhibit this process. Immunofluorescent study before grafting showed deposition of immunoglobulins and complements in hair follicles in both normal and affected scalp. However, a more striking deposition was noted in the affected skin. Deposition of immunoreactants after grafting was observed only after injection of patients' serum and not normal one. These experiments indicate that deposits of immunoreactants in hair follicles has no primary pathogenetic significance in AA/AU and that injections of serum obtained from patients with AA/AU did not have any affect on the hair growth process in our murine model.

EFFECT OF CYCLOSPORINE A ON THE REGULATION OF Ia ANTIGEN IN VIVO. Amos Gilhar, Thomas Pillar, Bedia Assay, and Shmuel Eidelman, Skin Research Laboratory, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Since many skin diseases characterized by positive Ia keratinocytes show improvement with cyclosporine (CyA) therapy, the purpose of this study was to determine whether CyA alters keratinocytes expression. Nude mice were injected with normal mouse serum to induce keratinocytes expression of Ia antigen. The injected mice were divided into 4 groups: one was treated with oral CyA; the second was treated topically with CyA twice a day on the ventral surface of the right ear; the third was treated topically with olive oil in the same location of the second group, and the fourth was injected with nude mouse serum. The mice were treated during the first 10 days after the injections. On day 10, epidermal sheets were analyzed for Ia expression. Analysis was made by an indirect immunoperoxidase staining method using monoclonal antibodies specific for Ia determinants. Quantitation of the number of LC was analysed on epidermal sheets using immunodiagnostic reagents, anti HLA-DR and surface ectoenzyme, ATPase. A significant reduction of Ia-positive keratinocytes was noted in the oral CyA group vs. topical and olive oil groups (64.85±29.89 vs. 20.11±18.68 percents respectively, $p<0.01$). In the second experiment mice were treated as in the first for 10 days, but treatment was started only on day 10 after injections. The results showed that CyA failed to down-regulate Ia expression. Topical and systemic CyA did not modify LC population. We conclude that systemic administration of CyA reduced Ia induction by epidermis of nude mice given normal serum.

PEPTIDERGIC NEURONS REGULATE MURINE DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTIONS. G.Girolomoni, P.R. Bergstresser, and R.E. Tigelaar. Dept. of Dermatology, UT Southwestern Medical Center, Dallas, TX.

Capsaicin (CAPS) is a neurotoxin that leads to depletion of neuropeptides (NP) in primary sensory neurons (C and A-δ type) involved in nociception and neurogenic inflammation (NI). We employed CAPS to explore the role of these neurons on the expression of DTH reactions in mice. BALB/c mice (6-8 wks) injected s.c. with CAPS (100 mg/kg) showed dramatic and protracted (>10 wks) impairment of sensory nociceptive function, with >80% reduction in the eye-wiping response to topical CAPS. Ear swelling to topical CAPS was reduced by >90%. Early (<4 hr) but not late (24 hr) ear swelling to DNFB in nonimmune mice was reduced >60% by CAPS pretreatment, suggesting that NI is a major component of the early irritant reaction to DNFB. In contrast, CAPS-pretreated (CAPS-ptd) mice exhibited enhanced DTH reactions to SRBC (>20%) and contact sensitivity (CS) to oxazolone (133%) and DNFB (38-64%). Adoptive transfer experiments showed that CS augmentation was not due to generation of increased numbers and/or activity of effector T cells, but more likely related to changes in their ability to enter elicitation sites. Consistent with this possibility are studies showing increased migration of ^{51}Cr-labelled, antigen-nonspecific lymph node cells in DNFB-immune, CAPS-ptd mice and histologic studies showing a more prominent cellular infiltrate in CS elicitation sites of CAPS-ptd mice.

These results suggest that peptidergic neurons, via release of NP, have a striking regulatory role in the expression of DTH reactions. This is the first report that the net effect of such peptidergic neurons on the late (cellular) phase of DTH and CS responses is *suppressive* (i.e. their impairment results in augmented reactions).

GAMMA-INTERFERON INHIBITS THE RETRACTION OF COLLAGEN LATTICES BY NORMAL AND SCLERODERMA SKIN FIBROBLASTS. P. Gillery, H. Serpier*, B. Kalis*, F.X. Maquart. Laboratory of Biochemistry, CNRS URA 610, and * Department of Dermatology, Faculty of Medicine, Reims, France.

Whereas several papers were devoted to the effects of γ-interferon (γ-IFN) on fibroblast monolayer cultures, no data were published about its effects on the same cells cultivated in 3-dimensional collagen lattices. We investigated the effects of recombinant γ-IFN (Boehringer - Ingelheim, France) on the retraction of collagen lattices by normal and scleroderma skin fibroblasts. In the case of normal fibroblasts, a significant inhibition of lattice retraction was observed from the first day of culture for a concentration of γ-IFN as low as 10 U/ml. Increasing the concentration provided stronger inhibition. For 10000 U/ml, the retraction was nearly completely stopped. In the case of scleroderma fibroblasts, the inhibition was significant at the concentration 10 U/ml from the second day of culture. A concentration-dependent effect was also found. The inhibiting effect of γ-IFN on the final intensity of the retraction was of the same order of magnitude in normal and scleroderma fibroblasts. No cytotoxicity was observed in both cases.

These results demonstrate that the retraction of collagen lattices by fibroblasts may be inhibited by γ-IFN. Previous data from our laboratory showed that lattice retraction is increased in severe forms of scleroderma. The relationship between these findings and the potential role of γ-IFN in vivo needs further investigation.

IDENTIFICATION OF TWO COLLAGEN-LIKE DOMAINS ON THE BULLOUS PEMPHIGOID ANTIGEN, BP180. G.J. Giudice, P. Elias, H.L. Squiquera and L.A. Diaz, Dept. of Dermatology, Med. College of Wisconsin, Milwaukee, WI.

Bullous pemphigoid (BP) is characterized by subepidermal vesicles and autoantibodies against hemidesmosomes (HD). Two epidermal antigens, BP240 and BP180, are commonly recognized by BP autoantibodies. We have recently isolated a 1.6 kb BP240 cDNA and a 1.0 kb BP180 cDNA from a human keratinocyte lambda gt11 cDNA library. Northern blot analysis demonstrated that BP240 and BP180 are encoded by distinct transcripts which are 8.5 kb and 6.0 kb in length, respectively. Nucleotide sequence analysis has revealed that a large portion of the BP180 cDNA, a stretch of 819 nucleotides, exhibits a significant homology with the collagen gene family. The deduced amino acid sequence of this region also shows a collagen-like structure with very high glycine and proline content (31% and 23%, respectively) and glycine appearing at every third position throughout most of this region. A non-collagen stretch of 14 amino acids subdivides this region into two collagen-like domains, with lengths of 240 and 30 amino acids. Preliminary studies indicate that incubation of the BP180 cDNA-encoded fusion protein with collagenase results in proteolysis and a loss of reactivity of the fusion protein with BP autoantibodies. We speculate that the collagen domains of the BP180 antigen may interact with an extracellular matrix component, e.g., laminin, fibronectin, collagen IV/VII or a proteoglycan, providing the molecular framework for the adhesive interaction between the epidermal HD and the dermis.

ABNORMAL CIRCULATING MONOCYTES IN PROGRESSIVE SYSTEMIC SCLEROSIS. M. Girardi, P. Heald, M. Kelleher, M. Perez, and R. Edelson. Dept. of Dermatology, Yale Univ. School of Medicine, New Haven, CT

Progressive systemic sclerosis (PSS) is characterized by overproduction of connective tissue collagen along with organ infiltrates of lymphocytes, monocytes/macrophages, and fibroblasts. The pathogenic role of each cell type is unknown, but the response of this disease to photopheresis therapy suggests circulating cells play a role. The BE-2 antigen is a 78,000kD surface protein which appears as a late activation antigen in T-cells and as a tumor associated antigen on malignant T-cells in cutaneous T-cell lymphoma (CTCL) patients. Neither lymphocytes or monocytes taken from normal donors exhibit BE-2 expression. Ficoll isolated mononuclear cells from PSS patients were analyzed by flow cytometry for the expression of BE-2 antigen. Of the 7 patients studied, all 7 were found to have abnormally elevated expression levels of BE-2 positive cells (range of 10 - 25%). These values were compared to an accepted clinical staging system for PSS and a significant correlation ($R = 0.80$) was noted between BE-2 levels and skin thickening from the disease. The use of two-color fluorescence staining methods with BE-2 (FITC) along with phycoerythrin-conjugated CD4 or MO-2 (Coulter) demonstrated that the abnormal expression of BE-2 is in the monocyte compartment with BE-2(+)/ MO-2(+) cells. CD4(+) lymphocytes were BE-2(-). These results suggest that PSS is a disease characterized by circulating activated monocytes.

LASER-INDUCED PHOTOTOXIC EFFECTS OF ALUMINUM PHTHALOCYANINE TETRASULFONATE ON HUMAN SQUAMOUS CELL CARCINOMA AND MALIGNANT MELANOMA CELLS *IN VITRO*. E. Glassberg, L. Lewandowski, R. Berne, G.P. Lask, and J. Uitto. Jefferson Med. College, Philadelphia, PA.

Photodynamic therapy (PDT) utilizes photosensitizing agents, such as aluminum phthalocyanine tetrasulfonate (AlPcS), to induce selective cytotoxicity in malignant tissue in vivo and in vitro, when exposed to the appropriate light energy. Human malignant melanoma (WM164) and squamous cell carcinoma (SCC-25) cells were tested in vitro to determine their sensitivity to PDT using AlPcS as the photosensitizer. AlPcS was tested at concentrations of 3 or 10 μg/ml, with energy densities in the range 0.1-16 J/cm^2 generated by a continuous wave tunable dye laser at 675 nm. Test parameters examined included [^{3}H]thymidine uptake (a measure of DNA synthetic activity) and [^{35}S]methionine incorporation (a measure of protein synthetic capacity). AlPcS alone, upto 25 μg/ml, incubated with cells for 16 hours caused no toxicity to either cell line. Laser light alone had no effect, upto 4 J/cm^2, on melanoma cells, and upto 16 J/cm^2, on SCC-25 cells. Combination of energy density at 1.0 J/cm^2 with 3 or 10 μg/ml of AlPcS inhibited DNA synthesis in WM164 cells by 71% and 91%, respectively. Using the same parameters, protein synthesis was inhibited by 77% and 96%. An energy density of 4.0 J/cm^2 with 3 or 10 μg/ml of AlPcS inhibited DNA synthesis in SCC-25 cells by 47% and 98%, respectively; protein synthesis was inhibited by 84% and 98.5%. The toxicity was both energy density-dependent and AlPcS concentration-dependent in both cell lines. Thus, AlPcS is an effective photosensitizer for these two human skin cancer cell lines in culture.

CHILD SYNDROME: INVOLVED-SKIN FIBROBLASTS FAIL TO PROLIFERATE IN RESPONSE TO INTERLEUKIN 1a (IL-1a) DUE TO AN EXCESSIVE INDUCTION OF PROSTAGLANDIN E_2 (PGE_2) SYNTHESIS. Marc E. Goldyne and Laidler Rea, Depts. of Dermatology and Medicine, V.A. Medical Center and University of California San Francisco, CA.

CHILD Syndrome is an acronym for Congenital Hemidysplasia with Ichthyosiform erythroderma and Limb Defects. We recently reported major differences in PGE_2 synthesis and proliferative rates between the involved-skin (I) and uninvolved-skin (U) fibroblasts (J. Clin. Invest. 84:357, 1989). Because IL-1a stimulates fibroblast proliferation as well as cyclooxygenase (enzyme initiating PGE_2 synthesis) generation among some fibroblast populations, we studied the proliferative effects of IL-1a on the two populations of CHILD Syndrome fibroblasts. I and U fibroblasts were individually cultured over 10 days in the presence and absence of 3 U/ml IL-1a. The U fibroblasts proliferated in response to IL-1a and only generated very low levels of PGE_2 at the end of the 10 day incubation. The I fibroblasts failed to proliferate in response to IL-1a and generated levels of PGE_2 50-100 fold higher than those found in the supernatants of the U fibroblasts. Whereas indomethacin had no effect on the proliferation of the U fibroblasts either in the presence of absence of IL-1a, it succeeded in establishing a significant proliferative response in the I fibroblasts following exposure to IL-1a. Adding exogenous PGE_2 was able to suppress the proliferative rate of the I fibroblasts. Thus, the lack of a proliferative effect of IL-1a on the I fibroblasts is due to the suppressive effect of excessive PGE_2 synthesis induced by IL-1a.

L-TRYPTOPHAN INGESTION ASSOCIATED WITH EOSINOPHILIC FASCIITIS. Marsha L. Gordon[1], Mark G. Lebwohl[1], Robert G. Phelps[1], Steven R. Cohen[1,2], and Raul Fleischmajer[1], Departments of Dermatology, Mount Sinai Med. Ctr., New York[1], and Beth Israel Med. Ctr., New York[2].

Recently, the ingestion of L-tryptophan has been associated with the Eosinophilia-Myalgia Syndrome (EMS), which is characterized by eosinophilia, myalgias and other symptoms such as cutaneous edema and skin induration. We are reporting 11 patients who fulfill the criteria for eosinophilic fasciitis, all of whom have taken L- tryptophan. Several brands in doses from 200-4,002 mg daily had been taken for 9 weeks to 11 years. 10 patients were female. All patients had eosinophilia, ranging from 15% to 88% of the WBC differential, and skin tightness. 8 patients initially complained of myalgias; the other 3 had difficulty distinguishing myalgias from arthralgias and cutaneous tenderness. Other symptoms were malaise, edema, pruritus, weakness, arthralgias, rashes, cutaneous burning and muscle spasms. Serologic profiles including anti-centromere antibody, topoisomerase and RNP were negative in the 6 patients that were tested. Pathology was most often consistent with eosinophilic fasciitis or scleroderma. It appears from this study that the clinical picture of eosinophilic fasciitis is similar to that described for EMS. We hypothesize that a subclinical impairment in tryptophan metabolism coupled with an increased intake of tryptophan may be the basis for this syndrome. This paper raises the question that derangements in the metabolism of tryptophan may play a role in sclerotic diseases.

IMMUNE SENSITIZATION AGAINST AUTOLOGOUS SKIN ANTIGENS IN POLYMORPHOUS LIGHT ERUPTION. R. González-Amaro, L. Baranda, J.F. Salazar-González, C. Abud-Mendoza, and B. Moncada. Department of Immunology, School of Medicine, San Luis Potosí, S.L.P., México.

Polymorphous light eruption (PLE) is an inflammatory skin disease induced by exposure to ultraviolet B light (UVBL). To further insight into the pathogenesis of PLE, we studied 9 PLE patients, as well as 6 healthy individuals. Two skin biopsies were obtained from each individual, one from a previously UVBL irradiated (IR) skin, and another one from an unirradiated (UR) skin zone. A keratinocyte (KE) cell suspension and/or skin homogenate were prepared from each biopsy. Autologous cultures were run mixing peripheral blood mononuclear cells (PBMNC) plus IR or UR skin homogenate, and PBMNC plus IR or UR KE suspension. Autologous mixed lymphocyte reaction (AMLR) was also performed. Cell proliferation was assessed by a ^3H-thymidine incorporation assay. Stimulatory cells (KE or non-T cells) were previously inactivated by mitomycin-C treatment. We found that the response of PBMNC to UR KE or UR skin homogenate was similar in both patients and controls. However, PBMNC from PLE patients showed a significative increased proliferative response to both IR KE or IR skin homogenate. Such phenomenon was not observed in controls. Cell proliferation in AMLR was similar in patients and controls. Our results indicate that UVBL increases the stimulatory capability of PLE KE in an unidirectional mixed culture with autologous PBMNC, suggesting that an immune sensitization against autologous UVBL-modified skin antigens ocurrs in PLE. Our data further support that PLE is an immune mediated disease induced by UVBL.

THE SEVERE COMBINED IMMUNODEFICIENT MOUSE (C.B.-17 SCID): A NEW MODEL FOR HUMAN SKIN CANCER INVESTIGATION. JB Goslen, MR Charley, MD Tharp, JS Deng, B Jegasothy Dept. of Derm., Univ. of Pittsburgh, PA

We have previously reported the establishment of cutaneous T cell lymphomas in SCID mice. The purpose of this study was to compare the relative abilities of SCID versus another immunodeficient mouse strain (C57/BALB/c beige-nude) to support a variety of transplanted human cancers. Tumors of different histologic types [basal cell carcinomas (4), laryngeal SCC (1), dermatofibrosarcoma protuberans (1), fibrosarcoma (1), malignant pilar tumor (4)] were transplanted subcutaneously in beige-nudes. Four BCCs were transplanted into SCIDs, three by split thickness surface skin grafting and one by subcutaneous implantation. The course of tumor growth was then assessed by direct measurement and histologic sampling weekly for up to 6 months. All tumors transplanted into beige-nudes underwent regression during the first 4-8 weeks. Thereafter, only tumors with aggressive initial histopathologic features (SCC, malignant pilar tumor, fibrosarcoma, and DFSP) demonstrated regrowth and progression. BCCs implanted in these animals did not reappear during the course of the study. Split thickness BCC grafts transplanted onto SCIDs developed surface crusting/necrosis resulting in loss of the tumor-bearing areas of the grafts, despite retention of the dermis and eventual re-epithelialization. However, BCC transplanted subcutaneously in the SCID mouse did not regress and when biopsied at 4 months still demonstrated the original BCC histopathology. In conclusion, although the beige-nude may be an acceptable model for certain cutaneous malignancies, the SCID is superior for the study of BCCs.

THE SEBACEOUS GLAND DUCT AS THE SOURCE OF THE SEBOLEMMAL KERATINOUS CASING OF THE SEBUM PLUG. A. Gonzalez-Serva, Departments of Pathology, Carney Hospital and V.A. Hospital (Boston Univ.), Boston, MA.

Sebaceous duct keratin is not shed perpendicularly in situ, but migrates forward as a thin and compact wavy sheath, or tubular casing, around the sebum. Random and serial sagittal sections with persistent and well preserved sebolemmal sheaths are presented.

Van Scott & MacCardle (1955) recognized hyperkeratotic ductal changes and a keratinous plug in the follicular neck in early acne, but the infundibularly located, yet not infundibularly derived comedo was not completely elucidated. In normal vellus pilosebaceous units, there is a wavy sheath of sebolemmal keratin, often pierced by a hair shaft (sebo-pilar channel). Terminal hair follicles have two separate keratinous channels, one solely pilar and another one (sebolemmal) for the sebum.

In acne vulgaris, turbulence at the vellus infundibulum may misorient an inherently weak sebo-pilar channel with a possibly hyperlipidized sebolemmal sheath. Delamination and disorganization of the sheath, as well as compaction of the stagnant plug ensues (comedo). This is followed by infundibular dilatation, appositional wall thinning and rupture. These later events induce only the inflammatory phase, but not the entire cycle of acne, at a falsely infundibular location.

TUMOR ANTIGEN PRESENTATION BY MURINE EPIDERMAL CELLS. S. Grabbe, R. Nazareno, S. Bruvers and R.D. Granstein, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Epidermal Langerhans cells (LC) can present antigen for CD4-dependent immunity in a number of systems and it has been hypothesized that they play a role in immunity to epidermal malignancies. This study examined epidermal cells (EC) for the ability to present tumor associated antigens (TAA) for in vivo tumor immunity. A/J ($H\text{-}2^a$) EC were prepared and enriched for LC by treatment with anti-Thy 1.2 + C. EC were then cultured for 12 h in 50 U/ml of GM-CSF, washed, cultured for 2 h in a solution of soluble S1509a fibrosarcoma (syngeneic to A/J) tumor cell fragments (TF), extensively washed and then injected s.c. into each of several A/J mice (1 to 2 x 10^5 EC/mouse). Control mice were injected with A/J EC treated identically but not pulsed with TF, BALB/c ($H\text{-}2^d$) EC pulsed with TF, or BALB/c EC alone. Immunization was repeated weekly for 2 weeks and 1 week after the last immunization mice were injected s.c. with 2 x 10^6 live S1509a cells. Tumor growth was then scored over time. Tumor volume was significantly reduced in mice immunized with A/J EC + TF compared with the other groups [3.5 $\pm$ 1.9(SEM)mm^3 for A/J EC + TF vs. 53.0 $\pm$ 20.0 for A/J EC ($p<0.04$), vs. 34.0 $\pm$ 13.5 for BALB/c EC + TF ($p<0.05$), vs. 33.0 $\pm$ 11.0 ($p<0.03$) for BALB/c EC at day 8 after tumor challenge, for example]. A preliminary experiment in which EC were depleted of I-A-bearing cells with anti-$I\text{-}A^k$ + C prior to pulsing with TF indicated that I-A-bearing cells were responsible for immunization in this system. Murine EC appear capable of presenting TAA for in vivo immunity.

TREATMENT OF KELOIDS WITH RECOMBINANT HUMAN GAMMA INTERFERON. R.D. Granstein, T.J. Flotte, T. Anderson, A. Haas, E. Unemori*, H.S. Jaffe*, and E.P. Amento*, Department of Dermatology, Harvard Medical School, Boston, MA and *Genentech, Inc., So. San Francisco, CA.

Gamma interferon (IFN-γ) inhibits collagen synthesis in vitro and in animal models in vivo. Therefore, recombinant human IFN-γ (rHuIFN-γ) was examined for its ability to modify keloidal scarring. Subjects were treated by injection of either 0.01 or 0.1 mg of rHuIFN-γ into one lesional site and diluent alone into another 3 times per week for 3 weeks. Lesions were measured prior to beginning therapy and weekly thereafter. Biopsies from treated and control sites were obtained 3 days after the final injection. Of 8 subjects studied, 6 had a reduction in size at the treated site with a mean reduction in height for all 8 subjects of 30.4 ± 8.1(SEM)% versus -0.7 ± 0.7% for control sites (p=0.002). Treated lesions exhibited both epidermal and dermal changes. The epidermis had thinned suprapapillary plates, compact hyperkeratosis, focal or diffuse parakeratosis, exocytosis of lymphocytes and an increased quantity of mucin. These changes are reminiscent of those seen in early psoriasis. The dermis contained a diminished quantity of thickened collagen bundles and active fibroblasts and an increased number of inflammatory cells and quantity of mucin. Of interest, rabbit antisera against active but not latent transforming growth factor β showed increased staining of treated specimens compared to controls (N=3). These results support the concept of using IFN-γ in the treatment of abnormal fibrosis. Dose-ranging studies are needed to establish whether IFN-γ can fulfill a clinical need in the treatment of keloids.

GAMMA-INTERFERON INDUCED RESISTANCE OF MELANOMA CELLS TO HUMAN LAK CELLS IS NOT MEDIATED BY ICAM-1 OR LFA-1. J Greenwald and RS Kalish, Dept. of Dermatology, Univ. of Minnesota, Minneapolis, MN

Gamma-interferon (INF) both increases the resistance of many tumor cells to killing by lymphokine activated killer cells (LAK) and increases the expression of ICAM-1 on many cell types. LFA-1/ICAM-1 interactions are not required for LAK cell cytotoxicity. The possibility that ICAM-1/LFA-1 interactions inhibit LAK cytotoxicity was tested. Human LAK cells were generated by incubation of PBMC with 100 U/ml of recombinant IL2 for 4 days. The ability of these LAK cells to kill SK-Mel 28 human melanoma cells and WS1 normal human fibroblasts was measured in a 4 hour 51Cr release assay. Incubation of SK-Mel with 500 U/ml of INF for 24 hours increased mean ICAM-1 expression (95% positive) from a mean linear channel fluorescence (MCF) of 70 to 155. Similar INF treatment of WS1 cells boosted ICAM-1 expression from 50% to 92% positive and increased MCF from 169 to 218. Pre-treatment of targets with 500 U/ml of INF for 24 hours decreased kill of the targets by LAK cells. Inhibition of kill was not blocked by anti(LFA-1) (TS1/22) or anti(ICAM-1) (RR1/1), suggesting that LFA-1/ICAM-1 interactions are not responsible for INF induced resistance to kill. (PERCENT CYTOTOXICITY AT (50:1) E:T RATIO)

	SK-MEL	SK-MEL+INF	WS1	WS1+INF
CONTROL	19%	15%	45%	42%
ANTI(LFA-1)	20%	15%	43%	33%
CONTROL	21%	11%	34%	18%
ANTI(ICAM-1)	18%	8%	22%	16%

A HISTAMINE RELEASING FACTOR IN SERUM OF CHRONIC URTICARIA WITH ANTI-IGE AUTOANTIBODY-LIKE PROPERTIES. C E H Grattan, D M Francis, M W Greaves, Institute of Dermatology, St Thomas's Hospital, London, UK.

The aetiology of chronic urticaria (CU) is unknown though an allergic cause is often assumed. A serum factor has previously been demonstrated by a wealing response to intradermal autologous serum. We aimed to characterise the serum factor using skin test reactivity *in vivo* and basophil histamine release *in vitro*.

24 CU patients (17 female, mean age 43.6 y), 5 patients with symptomatic dermographism (3 male, mean age 43.2 y) and 5 healthy controls (3 female, mean age 37.8) were recruited. Mean saline-corrected skin test weal volumes (mm3 +/- SD) at 60 min were 38.8 +/- 34.1 for CU patients, 0.8 +/- 3.3 for dermographics and -0.1 +/- 6.7 for healthy controls. Skin test reactivity of CU serum ultrafiltrates was confined to fractions >100 kD (n=8) with little or no response in either control group.

Sera of 9/24 CU patients elicited >10% histamine release (HR) above basal values from basophil leukocytes (BL) of one normal donor (mean 49.8 +/- 25.4%) at twofold dilutions. The response varied between basophil donors and was substantially lower in CU BL. The histamine releasing activity was only present in CU serum ultrafiltrate fractions >100 kD (n=8). HR in response to CU sera and goat antihuman IgE followed a similar time course and could be abolished by desensitisation of BL to A-IgE. HR activity was heat stable (56°C, 2 h, n=6) and was competitively inhibited by preincubation of sera with myeloma IgE. Preliminary protein G affinity column analysis indicates that the HR activity resides in the IgG fraction of at least some patients.

Peripheral blood cellular histamine (ng/ml +/- SD) of CU patients (21.5 +/- 12.4) was significantly reduced when compared to healthy controls (90.7 +/- 31.7). BL counts were also reduced in CU patients suggesting that the serum factor causes chronic degranulation .

Our results demonstrate a serological histamine releasing factor in CU with properties like an IgE autoantibody which may have an important pathogenetic role *in vivo*.

APPLICATION OF A RAPID, INTERNALLY CONTROLLED, COLORMETRIC ASSAY FOR TRANSIENT TRANSFECTION ANALYSIS TO MELANOCYTES J.M. Grichnik, and B.A. Gilchrest, USDA Human Nutrition Research Center at Tufts Univ, Boston, MA.

To study the transcriptional environment of the melanocyte/melanoma cell, we have successfully transfected primary cultures of human melanocytes and established a rapid, internally controlled assay system which employs the bacterial genes encoding β-glucuronidase and β-galactosidase. We have used $CaPO_4$ mediated transfection to co-transfect the DNA transcription promoting regions of SV40 (late) or β-actin linked to β-glucuronidase (generous gift of A.T. Sands) with a β-galactosidase expression vector transcriptionally driven by the rous sarcoma virus long terminal repeat as an internal control. We have demonstrated that the transfected gene products can be rapidly harvested from transfected melanocytes and assayed in a single buffer of 0.1% Triton X-100, 0.1M sodium phosphate (pH 7.3), 1 mM $MgCl_2$, and 50 mM β-mercaptoethanol. The assay for enzyme activity was initiated by the addition of 2 mM 4-nitrophenyl- β-D-glucuronide or O-nitrophenyl- β-D-galactoside. The production of the nitrophenol derivatives from enzymatic cleavage by β-glucuronidase and β-galactosidase respectively was monitored using a Bio-Rad EIA 96 well plate reader with a 414 nm (blue) filter. The assay was found to be linear over a 50-fold range. The enzymes displayed marked specificity for their substrates and non-transfected melanocyte extract assays had negliable background activity. This rapid colormetric assay has marked advantages over the traditional chloramphenicol acetyl-transferase assay system.

CULTURED HUMAN KERATINOCYTE PROTEOLIPIDS CONTAIN H^+-PUMP COMPONENTS AS WELL AS A DIFFERENTIATION-RELATED SPECIES. Stephen Grayson and Sandy M. Sequeira, Department of Dermatology, University of California School of Medicine, San Francisco, CA.

Cultured human keratinocyte proteolipids (CHK-PL) have been isolated and purified from chloroform-methanol mixtures. These very hydrophobic integral membrane proteins are characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) into species of Mr ≈30, 26, 22, 17.5, 15, and 12 kD. The 12 kD band is further characterized into five bands of Mr 8, 6, 5, 3, and 2 kD by high resolution SDS-PAGE. We have been able to disperse and characterize the significant macroaggregate component of CHK-PL by treatment with trifluoroacetic acid, providing increased yields of individual species and enabling further comparative analyses of gel permeant species. Our efforts to determine cell regulatory functions for these membrane proteins have resulted in the characterization of one species (Mr 30 kD) as being differentiation related, and two species which bind dicyclohexylcarbodiimide (Mr 8 and 17.5 kD) as putative H^+-pump subunits. The 8 kD species is likely a plasma membrane or mitochondrial H^+-pump component, and the 17.5 kD apoprotein possibly of vacuolar origin. Monospecific polyclonal antibodies to these species (by Western blotting) will provide necessary immunolocalization data. Evidence for incorporation of vacuolar H^+-pumps into plasma membranes of osteoclasts and urinary bladder epithelium, may serve as a model for the acidification of epidermal extracellular compartments by lamellar bodies, facilitating barrier lipid processing and desquamation.

THE INFLUENCE OF ION EXCHANGE INHIBITORS AND TRANSFORMING GROWTH FACTOR-β (TGF-β) ON KERATINOCYTE/ LYMPHOCYTE INTERACTIONS. C.E.M. Griffiths, J.N.W.N. Barker, R.S. Mitra, B.J. Nickoloff. Dept. of Dermatology, Univ. of Michigan.

Gamma interferon (IFN-γ) is known to induce intercellular adhesion molecule-1 (ICAM-1) and HLA-DR (DR) on human keratinocytes (KCs). Previous work suggests that ICAM-1, but not DR, expression is a protein kinase C dependent event. We investigated, using a Ca^{++} channel blocker, verapamil; an ion exchange inhibitor, amiloride; and a calmodulin antagonist, W7, whether DR expression is dependent on the Ca^{++}/Calmodulin second messenger system. Also, the functional effect of these compounds on KC/lymphocyte adhesion events was studied.

A T-cell line (Jurkat) or Ficoll-Hypaque-separated lymphocytes were labeled with ^{51}Cr, activated with TPA (25nM, 30 min) and incubated with W7, verapamil, amiloride or anti-LFA-1 antibody, (as control inhibitor). Treated lymphocytes were then co-incubated for 45 min with IFN-γ-treated KCs grown on coverslips in KGM & adherent lymphocytes were counted. The effect of the same treatments on IFN-γ-induced KC expression of ICAM-1 and DR was studied. Following pre-treatment with the various agents, KCs were incubated together with inhibitor and IFN-γ (10 U/ml 48 hrs). KCs were trypsinized, stained for ICAM-1 or DR & analyzed by FACS.

Verapamil inhibited KC/lymphocyte adherence (equal results with both types of lymphocytes) in a dose-dependent manner with maximal inhibition, 97% at 1mM; compared to W7 at 200 μM, and amiloride at 2 nM which inhibited by 93 and 61%, respectively. DR but not ICAM-1 expression was reduced by all 4 agents in a dose-dependent manner with peak inhibition with TGF-β 12 ng/ml of 75.2 ± 18.5%; or verapamil 0.2nM, 35.2 ± 5.8%; or amiloride 0.2nM, 44.2 ± 19%; or W7, 20 μM, 53.7 ± 6.2% (n=4).

These results demonstrate that both KC DR expression and KC/lymphocyte ICAM-1/LFA-1 interactions are Ca^{++} dependent processes. The disruption of KC/lymphocyte adherence by Ca^{++} exchange inhibitors introduces a novel therapeutic option for inflammatory dermatoses.

PROTEIN SULFATION: A UNIQUE MECHANISM OF ACTION OF MINOXIDIL SULFATE. V.E. Groppi[1], B-A. Burnett[1], L. Maggoria[2], C. Bannow[2], H.A. Zurcher-Neeley[2], R.L. Heinrikson[2], H.J. Schostarez[3] and M.J. Bienkowski[1], Cell Biology[1], Biopolymer Chemistry[2] and Hairgrowth Research[3], The Upjohn Company, Kalamazoo, Michigan.

Minoxidil sulfate (MNXS), the biologically active metabolite of minoxidil, relaxes vascular smooth muscle and promotes the growth of hair. In this report we show that MNXS has the unique ability to nonenzymatically sulfate proteins. Using a rapid filter binding assay, we screened over 50 peptides to characterize the direct sulfation reaction in vitro. A series of analogs of Angiotensin 1 was also analyzed by HPLC and N-terminal sequence analysis. When taken together, these data indicate that MNXS sulfates N-termini and histidines in these model peptides. These data also indicate that tyrosine, serine and threonine are not sulfated by MNXS. Intact cell labeling studies indicate that, in general, the same sites are sulfated in vivo and in vitro. Pulse-chase experiments establish that the sulfate on protein is very stable in exponentially growing cells (half-life approximately 12 hr). These results are consistent with the well known, long duration of action of MNXS. Therefore, we conclude that MNXS causes unique post-translational modifications of proteins in target cells that appear to be central to the mechanism of action of MNXS. Furthermore, the data suggests that MNXS-dependent protein sulfation may have similarities to protein phosphorylation in modulating signal transduction pathways.

MELANOSOMAL CATALASE AS A POSITIVE REGULATOR OF PIGMENTATION. Ruth Halaban and Gisela Moellmann, Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Tyrosinase-negative albinism is due to a mutant tyrosinase (*TYR*) gene. No gene controlling tyrosinase-positive albinism has been identified. One candidate would be a human counterpart to the murine *brown* (*b*) locus. Mutations at the *b* locus in mice lead to a brown (*b/b*) or almost white (B^{lt}) coat color versus the wild-type black (*B*). As deduced from the respective cDNAs, the proteins encoded by the murine *TYR*- and *b*-loci are of similar size, with a membrane-spanning domain and conserved positions of histidine and cystein. The regions of homology suggest that the *b*-locus protein, like tyrosinase, is an oxidoreductase. Using wild-type and mutant murine melanocytes and a battery of antibodies, we show that only the *TYR* protein has tyrosinase activity and that the *b*-locus protein is a catalase. We also show that the previously identified melanosomal gp75 is the human counterpart of the murine *b*-locus catalase. In melanocytes from a tyrosinase-positive albino and from two non-albinotic, fair-complexioned blond individuals, tyrosinase levels are normal but levels of the *b*-locus catalase are lower than in pooled melanocytes from randomly chosen Caucasians. Because H_2O_2, the substrate of catalase, is known to destroy eumelanin and its precursors, we conclude that pigmentation in individuals with normal tyrosinase is controlled in part by enzymes that catalyze the decomposition of H_2O_2, that mutations in the melanosomal catalase may lead to tyrosinase-positive albinism, contribute to the gradations of skin and hair color among different ethnic groups, and constitute the basis for the overt expression of pheomelanin in red hair.

Evaluation of Commercial Poison-Ivy Hyposensitization Antigens By HPLC/MS Analysis. Jere D. Guin, M.D., Paul Lehman, M.S., Douglas Gage, Ph.D., and Thomas J. Franz, M.D., Department of Dermatology, University of Arkansas for Medical Sciences, and Dept of Biochemistry, Michigan State University.

The content of many commercial poison ivy/oak extracts has been questioned as content of alk(en)yl catechols is not revealed. Content of urushiol is important, however because of both safety and efficacy, and this should be standardized for a product so that results are reproducible. The most active components of urushiol are probably the diolefin and triolefin fractions which can vary not only between clones, but with the season and even in different parts of the same plant. For whole urushiol extracts, the minimum total dosage required to detect minimal alteration in sensitivity is 600-800mg with 2-2.5gm necessary for maximal improvement. Unfortunately, no one has evaluated the amount of diolefin (and/or triolefin) required.

We analyzed the commercial oral and injectable hyposensitization products for poison ivy still available by reverse phase HPLC. Catechol identification was confirmed by PB/MS. We found impressive content of alk(en)yl catechols in the single oral antigen, but very small amounts of antigen in the injectable products. The data obtained explain the reported lack of local reaction at the site of injection and the putative failure to confirm benefit with quantitative patch testing, despite positive anecdotal reports. The presence of cis-and-trans isomers in the urushiol standard and extracts raises the possibility of component specificity. Reverse phase HPLC analysis seems to be a convenient, effective method for screening marketed products, for both manufacturer and regulatory agencies.

A NEW HIGH MOLECULAR WEIGHT PROTEIN FROM FILIFORM PAPILLAE: PURIFICATION AND PARTIAL CHARACTERIZATION. Elizabeth Harris Hamilton and Edward O'Keefe. Department of Dermatology, University of North Carolina, Chapel Hill, NC.

Distinctive non-keratin proteins of specialized epithelial structures such as tongue papillae have not been purified previously. We have purified a novel protein from pig tongue which is present in filiform papillae but is reduced or absent in epidermis. Epithelium from pig tongue was homogenized and depleted of keratins in citric acid buffer, pH 2.6. The residual insoluble structures examined on SDS polyacrylamide gels contained a prominent 200 kilodalton doublet (200 kd), which was extracted with low ionic strength buffer (0.1 mM EDTA, pH 8.0), precipitated with ammonium sulfate, and eluted from a Mono Q column with a NaBr gradient in 4 M urea. The proteins of the doublet, of 190 and 200 kilodaltons, have nearly identical peptide maps and pIs (6.6-6.7). 200 kd is also soluble in buffer containing 1 M NaBr, in which it has a Stokes radius in excess of 10 nm. Crosslinking with disuccinimidyl suberate produces a large complex which fails to enter 3% SDS polyacryamide gels. The crosslinking behavior and large Stokes radius suggest an oligomeric protein with an extended asymmetric configuration, presumably a structural protein. Localization in pig tongue with antibody purified on 200 kd linked to Sepharose shows intense staining at the base and lower part of filiform papillae and minimal or absent staining in the epidermis. 200 kd is a candidate for a novel structural protein associated with filiform papillae.

DISRUPTION OF PROTEOGLYCAN SYNTHESIS BY β-XYLOSIDE. MORPHOLOGICAL EFFECTS ON STRATIFIED CULTURES OF HUMAN KERATINOCYTES. John G. Haggerty, Randolph H. Bretton, & Leonard M. Milstone. VAMC, West Haven, CT and Yale University School of Medicine, New Haven, CT.

In numerous cells, proteoglycans are involved in proliferation, as well as adhesion of cells to each other and to the basal lamina. Synthesis of proteoglycans is disrupted by β-xylosides, which prevent glycosylation of the core protein by serving as alternate substrate sites for glycosaminoglycan chain attachment. We have investigated the effects of p-Nitrophenyl-β-D-xylopyranoside (β-xyloside) on cultured keratinocytes. Confluent cultures were incubated for 7 days with β-xyloside(0.1 to 1.0 mM). These concentrations decreased cell-associated proteoglycans and increased, 5-8-fold, ^{35}S-sulfate labelled material in the medium. This increase was in the form of glycosaminoglycans, consistent with the known effects of β-xylosides. Treatment with up to 1 mM β-xyloside had no effect on protein synthesis or the numbers of attached and desquamated cells indicating that the ability of keratinocytes to proliferate or stratify was not affected, although thymidine incorporation into DNA decreased. There was a significant effect on the morphology of stratified cultures released from dishes with dispase prior to fixation. Increased β-xyloside in the medium dramatically decreased the cross-sectional thickness of the cell layer. This effect could be mimicked by a short incubation of the cells in 0.02 mM Cytochalasin D, which disrupts actin filament formation. Thus, functional proteoglycans may be necessary to maintain normal keratinocyte morphology by some interaction with cytoskeletal elements.

HUMAN EPIDERMIS PROCESSES BOTH IL-1α AND IL-1β INTO NOVEL MOLECULAR ISOFORMS. Craig Hammerberg, Gary Fisher, John J. Voorhees, and Kevin D. Cooper, Department of Dermatology, Univ. of Michigan, Ann Arbor, Michigan.

In contrast to cultured cells, the regulation of Interleukin-1 (IL-1) activity by intact normal and inflamed human skin is poorly understood. IL-1α is reduced in psoriatic epidermis, while a dysfunctional IL-1β, also present in normal skin, is increased in psoriatic lesions. In contrast to cultured keratinocytes, IL-1β is not sequestered as an inactive large precursor, but exists as a 15 kD processed molecule, and would thus be predicted to be active. The processed IL-1 molecules were separated according to their isoelectric points by Fast Protein Liquid Chromatography (FPLC) chromatofocusing using a Mono P column. IL-1α or IL-1β were detected in various fractions by using the appropriate ELISA for immunoreactivity or LBRM.33 cells for functional activity. Immunoreactive IL-1α molecules eluted around pH 5.0 while psoriatic immunoreactive IL-1β molecules eluted at three pH's (7.0, 6.0, and 5.0), all of which remained inactive functionally. Interestingly, the pI's of the IL-1β molecules were different from that of recombinant (r) human IL-1β or processed native monocytic IL-1β (pI 8.0). Immunoreactive psoriatic IL-1α and rIL-1α eluted at pH 5. In the functional assay, one peak of IL-1 activity was associated with the pI 5 immunoreactive IL-1α molecule, while two other peaks of IL-1 functional activity (pI 8.0 and 6.5) were associated neither with the immunoreactive IL-1β molecules nor the acidic immunoreactive IL-1α. Although unreactive with the ELISA monoclonals, experiments with various polyclonal anti-IL-1 antibodies demonstrated that the pI 8.0 and 6.5 IL-1 functional activity was neutralized only with anti-IL-1α antibodies. Thus, like the IL-1β molecules, the IL-1α molecules are also not identical to the immunoreactive or rIL-1α molecule (both pI 5.0). These data demonstrate that human skin in vivo can regulate IL-1 in a unique manner by processing IL-1β and IL-1α into novel forms not previously observed in cultured keratinocytes or in other organs. Differential processing of both forms of IL-1 in the epidermis suggest that epidermal IL-1 molecules may also have novel functions, especially in the case of the dysfunctional IL-1β.

EFFECT OF GLUTATHIONE DEPLETION ON SUNBURN CELL FORMATION IN THE HAIRLESS MOUSE. Katsumi Hanada*, Richard W.Gange*, Michael J.Connor**. *Wellman Laboratories of Photomedicine, Department of Dermatology, Harvard Medical School, Boston, MA and **UCLA School of Medicine, Department of Medicine, Division of Dermatology, Los Angeles, CA.

Cutaneous protection against UVB radiation damage by endogenous glutathione (GSH) was evaluated in the epidermis of the hairless mouse by measuring the influence of GSH depletion on sunburn cell (SBC) formation. Cellular GSH exerts anti-oxidant effects, and recent studies have suggested a role for oxygen radicals in the production of sunburn cells. Hairless mice (Skh/hr 1) received oral treatment with buthionine S,R-sulfoximine (BSO), an irreversible inhibitor of *r*-glutamylcysteine synthetase to deplete cutaneous glutathione; 24 hours later the ears were exposed to UVB radiation. The GSH levels in the epidermis and dermis were significantly reduced by BSO treatment, to 10-15% of control levels. SBC count in the ears of animals with and without BSO-treatment increased with UVB dose. Greater numbers of SBC were found in the ears of BSO-treated mice exposed to 15 or 20 mJ/cm^2 UVB, than in non BSO-treated mice exposed to same UVB doses. At higher UVB doses, there were no statistically significant differences between the groups. The results show that endogenous GSH provides measurable protection of the epidermis against injury by moderate UVB doses.

STIMULATION OF IL-8 IN A HUMAN KERATINOCYTE CELL LINE. Alexander Haslberger, Rene Blanka, Carolyn A. Foster, Miroslav Ceska, Anton Stütz, Ivan Lindley, Sandoz Forschungsinstitut, Vienna, Austria.

Infiltration of neutrophils into the epidermis is seen in various skin diseases. Therefore, we investigated the stimulation of the recently cloned, chemotactic neutrophil activating protein (NAP1/IL8) in the basal human keratinocyte HACAT cell line using northern blots and a highly sensitive double ligand ELISA. A dose-dependent expression of IL8 protein (0.04-2 ng/mL) was found 8 hrs after stimulating the confluent cells with IL1α (250, 25 U/mL) and TNFα (1000, 100, 10 U/ml). Additionally, IL8 protein concentrations were detectable after stimulation with IL3 and IL6 (200 U/ml). This was accompanied by a marked accumulation of IL8 mRNA 8 hrs after stimulation with IL1α, TNFα and IL3. LPS, PMA and Bradykinin did not induce IL8 expression although HACAT cells respond to these stimuli with accumulation of c-fos mRNA and synthesis of PGE_2. Dexamethason (5 µM) clearly inhibited the IL1α- and TNFα-induced^2 IL8 expression, whereas Cyclosporin A treatment (2 µM) had no significant inhibitory effect. These data show that the stimulation of IL8 in keratinocytes is induced by specific immune mediators whereas PMA and Bradykinin, both potent inducers of inflammatory reactions in the skin, are not capable of stimulating IL8 synthesis.

STIMULATION OF DNA SYNTHESIS IN PRIMARY MOUSE EPIDERMAL KERATINOCYTES AND CULTURED WHOLE FOLLICLES BY POTASSIUM CHANNEL OPENERS (PCOs). C. S. Harmon, J. Ducote and D. Lutz, Preclinical Research, Roche Dermatologics, Nutley, N.J.

It has previously been shown that minoxidil has a number of direct effects on cultured mouse epidermal keratinocytes and whole follicles, including stimulation of DNA synthesis. Since the pharmacological actions of minoxidil are thought to result from its conversion to minoxidil sulfate, which has PCO activity, we have studied the proliferative effects in these culture systems of a number of structurally distinct PCOs, including pinacidil, cromakalim and diazoxide. Epidermal keratinocytes from negnatal mice were plated in RPMI 1640 containing 10% FBS at 500,000/cm^2 and fed every 2-3 days with medium containing test compound. DNA synthesis was measured from ^{3}H-thymidine incorporation on day 8. Whole whisker follicles were isolated nonenzymatically by careful dissection from whisker pads excised from neonatal mice. Follicles were incubated in M199 medium containing 20% FBS, and ^{3}H-thymidine incorporation was measured over days 2-5 of culture. Pinacidil stimulated epidermal keratinocyte DNA synthesis to an extent (60% at 25 uM) comparable to that obtained for minoxidil, while cromakalim was less active.. In the whole follicle assay, comparable (40-80%) stimulation of DNA synthesis was obtained for minoxidil, cromakalim and pinacidil, while diazoxide showed greater activity. These results are consistent with the hypothesis that the hypertrichotic activities of PCOs such as minoxidil sulfate, diazoxide and pinacidil result, at least in part, from direct effects on follicular keratinocytes.

MURINE EPIDERMAL CELLS ARE CAPABLE OF GENERATING PORPHYRINS FROM δ-AMINOLEVULINIC ACID. Dan He, Evan Karas, Shigeru Sassa, and Henry W. Lim, Dermatology Service, New York VAMC, and Department of Dermatology, NYU School of Medicine, New York, NY; The Rockefeller University, New York, NY.

This study was designed to investigate the ability of epidermal cells to participate in heme biosynthesis. Epidermal cells, obtained from skin of Skh: HR-1 hairless albino mice by trypsinization, were incubated with heme precursor δ-aminolevulinic acid (ALA); porphyrins (PP) generated were measured spectrofluorometrically. The yield was 55.9 ± 4.1 x 10^{4}VEC (viable epidermal cells)/mouse. Viability of the epidermal cells, as determined by trypan blue exclusion, was 86.3 ± 0.8%. Using 2.0 mM ALA, PP production increased in a time-dependent fashion, from 1.2 ± 0.2 ng/10^{4}VEC at 2 hr, to 10.5 ± 1.0 ng/10^{4}VEC at 48 hr. In the absence of ALA, PP level was 0.6 ± 0.1 ng/10^{4}VEC. Dose response study (0.1 - 3.0 mM ALA) was performed using an incubation time of 24 hr. There was an ALA dose-dependent increase in the production of PP, reaching a plateau of 7.6 ± 1.0 at 0.6 mM ALA. CaMg EDTA (1.25 - 5.0 mM), a ferrochelatase inhibitor, enhanced PP production by 10 - 30%, suggesting that ferrochelatase was present in these cells. Therefore, this study demonstrated for the first time that murine epidermal cells possessed heme biosynthesic pathway, and suggests that these cells may be an useful model for the study of cutaneous porphyrin metabolism.

PRODUCTION OF MONOCLONAL ANTIBODY AGAINST A HIGH MOLECULAR WEIGHT DESMOSOMAL PLAQUE PROTEIN AND ITS cDNA CLONING. Takashi Hashimoto, Masayuki Amagai, Hironori Niizeki, Masashi Akiyama, Yoshio Inokuchi*, Nobuyoshi Shimizu* and Takeji Nishikawa, Departments of Dermatology and *Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

We have obtained a monoclonal antibody (MAb) using spleen cells of a mouse immunized with the bovine desmosome preparation. The MAb reacted with a protein of an approximate molecular weight of 680,000 in the extracts of normal human epidermis and cultured human and mouse keratinocytes. Immunogold electron microscopy revealed that the MAb reacted specifically with cytoplasmic attachment plaque of desmosome. We then constructed lambda ZAP II expression library utilizing mRNA extracted from Pam 212 cells which produced large amount of the 680kD protein. By immunoscreening we isolated 9 cDNA clones with the DNA inserts ranging from 2 to 4kb. Each clone produced the fusion protein reactive with the MAb even after in vivo excision of pBluescript from lambda ZAP II by f1 helper phage. Northern blot analysis detected two mRNA spieces of 12kb and 14kb.

The 680kD protein may be a new structural component of desmosomal attachment plaque. Further analysis of this protein may provide us with important new information on the molecular organization of the desmosome.

SPECIFIC SUPPRESSION OF T-CELL SURFACE ANTIGENS WITH ANTISENSE OLIGODEOXYNUCLEOTIDES. P. Heald, J. Latkowski, F. Gasparro, S-L Yan, R. Edelson. Yale Univ. Dept. of Dermatology, New Haven CT.

Specific T-cell surface protein regulation is a goal of dermatologic therapy. The gene sequence of surface proteins can be used to design oligodeoxynucleotides (oligos) which are complementary to the DNA or RNA for a given protein. 15 base oligos with a phosphothiorate backbone were prepared complementary to the first 15 bases for amino acids in the RNA sequence for CD1 protein. In addition, control 15 base random sequence oligos were prepared. The T-cell line MOLT-4 was incubated with these oligos and the specific effects on CD1 expression were compared to nonspecific effects on CD7 expression. Cell surface expression of CD1 was unchanged at 12 hours, suppressed by 17% at 18 hours with a maximum of 35% suppression at 24 hours. There was no inhibition with the random sequences. By 48 hours the cells recovered from oligo induced inhibition. The maximal inhibition was achieved with cells at 2x10^6 cells/ml and 10uM of oligo complementary to the RNA. The inhibitory effect of the oligo was potentiated when cell division was arrested with colchicine. Under these conditions, CD1 expression was decreased by 63% when compared to colchicine treated controls. The inhibition was specific with no effect of the CD1 antisense oligo on CD7 expression and with no effect noted of random 15 base oligos on CD1 expression. Cell surface proteins have the potential to be therapeutically regulated based on the specificity of their genetic sequence.

THE EFFECTS OF COMBINATIONS OF GROWTH FACTORS ON EPITHELIALIZATION IN AN EXPLANT CULTURE MODEL: IMPLICATIONS FOR WOUND HEALING. PA Hebda, Ph.D., Dept. of Dermatology, Univ of Pittsburgh School of Med., Pittsburgh, PA 15261

Explants provide an in vitro model for epidermal wound healing. Epidermal cell outgrowth from explants resembles wound epithelialization in the ordered sequence of migration, mitosis and maturation (keratinization) of the epidermal sheet. No single factor can stimulate and support epidermal outgrowth from partial thickness skin explants without the presence of at least a low concentration of serum (>0.5%), suggesting that more than one growth factor is needed. The explant model was used to evaluate a number of growth factors, and different biological effects on epidermal cell outgrowth were observed. Combinations of growth factors with complementary functions were also tested to look for additive and synergistic effects. Recently, a unique combination of growth factors was found that replaced serum. Transforming growth factor-beta (TGF-beta) and insulin-like growth factor (IGF) in RPMI 1640 medium stimulated and supported epidermal cell outgrowth in the absence of serum or other supplements. The unique bifunctional role of TGF-beta in regulating epithelial cells (stimulation of migration, inhibition of mitosis) may be essential in the special environment of injured epidermal tissue. The effects of IGF may also be unique compared with the effects of other epithelial mitogens, including epidermal growth factor and fibroblast growth factor. The results indicate possible roles for TGF-beta and IGF as endogenous signals for initiating epidermal wound healing.

POTENCIES OF PROTEIN KINASE C INHIBITORS IN A CELL FREE SYSTEM ARE STRICTLY CORRELATED TO THEIR INHIBITORY PROPERTIES IN KERATINOCYTE CULTURE. Lutz Hegemann°*, Bernd Bonnekoh°, Bernard Schmidt*, Lucio A. A. van Rooijen*, Jörg Traber* and Gustav Mahrle°
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Phospholipid- and calcium dependent proteinkinase (PKC) was shown to play a key role in cell proliferation (Nishizuka, Nature: 308, 693, 1984). Recently it was found that PKC differed in keratinocytes derived from normal and psoriatic skin (Inohara et al., Arch. Dermatol. Res.: 172 (1), 146, 1987). In the present study we compared the potencies of known PKC inhibitors (PKC-I) in both, the cell free system and on protein synthesis and cell growth of keratinocyte cultures. Therefore PKC was partially purified from male Wistar rat brain and assessed by histone phosphorylation. For cell cultures we used human keratinocytes (HaCaT-cell line, Boukamp et al., J. Cell. Biol.: 106, 761, 1988). The effects of PKC-I were monitored by measuring ^{3}H-thymidine- and ^{14}C-amino acid incorporation as well as total protein content and cytotoxicity by LDH release. The rancing of PKC-I concerning their inhibitory properties in the cell free system and in the cell culture assay on all parameters detected was identical; staurosporine was the most potent inhibitor (IC_{50}-value: ca. $5x10^{-8}$ M), much more potent than phenothiazine derivatives and other dual calmodulin/PKC-I (IC_{50}-values from $5x10^{-4}$ M to 10^{-5} M). The IC_{50}-values in the cell free assay and the culture system were highly correlated (r=0.92). These results may stimulate further studies on the antipsoriatic potency of PKC inhibitors.

Stimulation of Epidermal and Dermal Wound Healing by Kaposi Sarcoma-Derived Fibroblast Growth Factor. PA Hebda*, EP Brady*, N Wolfman#, J. Stoudemire#, D Rogers#. *Dept of Dermatology, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; #Genetics Institute, Cambridge, MA

Kaposi sarcoma-derived fibroblast growth factor (K-FGF) is a member of the homologous family of fibroblast growth factors which also includes acidic and basic FGF. K-FGF was first isolated from cultured tumor cells and is now available in a highly purified recominant form. K-FGF is believed to play a role in development since message and protein levels are high in fetal tissue, but both decrease around the time of birth. Although it is not known whether all FGFs interact with a single cellular receptor, K-FGF exhibits some distinctive biologic effects. For this reason and because it may be a developmental factor as well as a growth factor, we evaluated the effects of K-FGF on wound healing using porcine wound models. Our results show that K-FGF significantly stimulated 1) epidermal healing in the range of 100 ng-10 ug/wound and 2) regain of tensile strength at 10 ug/wound. These effects were achieved after a single application of K-FGF in an aqueous buffer on the day of wounding. K-FGF maximally stimulated epidermal healing (18% stimulation) at 10 ug/wound (0.5mg/ml) but retained partial activity (11% stimulation) at 100 ng/wound (5 ug/ml). Its ability to stimulate epithelialization at such a low concentration suggests a relatively high affinity for the FGF receptor and/or resistance to inactivation in the wound milieu. Because of its stability, its potency to normal wound healing and its ability to stimulate in a single application, K-FGF has great potential as a clinical treatment for various types of wounds.

A NEW MODEL FOR A MODIFIED LYMPHOCYTE TRANSFORMATION TEST IN THE DIAGNOSIS OF ALLERGIC DRUG REACTIONS: GLUTATHIONE ACTS AS AN AMPLIFYER OF MITOGEN- AND ANTIGEN-INDUCED LYMPHOCYTE ACTIVATION. M. Hertl, F. Jugert, H. F. Merk, Department of Dermatology, University of Cologne, J. Stelzmannstr. 9, D-5000 Köln 41, West-Germany.

In the detection of drug hypersensivity reactions the lymphocyte transformation test (LTT) has proven a useful in vitro-method. For the purposes of this assay peripheral blood mononuclear cells (PBMC) of drug allergic patients are incubated with the allergen in short term cultures. Allergen specific lymphocyte proliferation is determined by the uptake of tritiated thymidine. Studies in penicillin allergic patients have shown that this molecule, which needs no further enzymatic metabolism to be immunogenic, can act as a strong stimulus for the in vitro-activation of specific lymphocytes. Most other allergens undergo a cytochrome P-450 dependent degradation prior to being efficient inducers of specific lymphocyte responses in sensitized patients. In 13 of 25 patients who were allergic to drugs such as phenobarbital, chloroquine, and promethazine lymphocyte proliferation occurred only after the preincubation of the employed allergen with murine microsomes. Murine microsomes alone did not elicit an increased lymphocyte proliferation. A new approach is the amplification of a drug specific response by adding glutathione (GSH) to short term lymphocyte cultures. Liang et al. have shown that GSH enhanced the proliferation of interleukin-2 (IL-2) dependent T cell lines by increasing the amount of bound and internalized IL-2 (JBC 264:1319, 1989). In our experiments adding of GSH to mitogenesis assays with PHA enhanced PBMC proliferation by 66 - 80% of the control (PBMC + PHA: 7964 cpm; PBMC + PHA + GSH: 14018 cpm). The optimal GSH concentration used was 0.5 mM, which caused a similar increase in PHA-dependent PBMC proliferation when added 24 or 48 hours after the setup of the culture. PBMC proliferation to tetanus toxoid was enhanced by 100% (PBMC + TT: 5704 cpm; PBMC + TT + GSH: 11169 cpm) when 0.5 mM GSH was added after 24 hours of culture. This augmenting effect of GSH is helpful to increase the sensitivity of the LTT in the detection of adverse reactions to drugs that are only weak stimulators of in vitro lymphocyte responses.

INTERLEUKIN-1α INDUCES BM40/SPARC IN DERMAL MICROVASCULAR ENDOTHELIAL CELLS M Heckmann, M Karasek, P Cline, and T Krieg, Depts. of Dermatology, Stanford University, Stanford, CA, and Ludwigs-Maximilians-University, Munich, FRG.

Inflammation and tissue remodelling as seen in various skin diseases and wound healing is associated with the release and activity of cytokines, such as interleukin-1 (IL-1). Originally ascribed to monocytes/macrophages, IL-1 is now recognized as a pleiotropic cellular mediator which can be released from epidermal and dermal cells, including keratinocytes, fibroblasts and endothelial cells. In turn, IL-1 exerts a broad spectrum of effects on these cells, generally resulting in an inflammatory condition.

We have investigated the effect of IL-1α and phorbol myristate acetate on human dermal microvascular endothelial cells focussing on the regulation of BM40/SPARC, a potent, Ca^{2+} binding matrix protein typically found in tissues characterized by increased cellular turnover and basement membrane formation. The recent completion of the human cDNA sequence confirmed the identity of BM40 with SPARC and osteonectin, facilitating the determination of gene transcription of this novel protein. Cell cultures of human microvascular endothelial cells were initiated from foreskin tissue and grown in cAMP and serum supplemented Iscove's medium. mRNA levels of BM40/SPARC were analyzed by Northern blotting and compared to ß-actin, a constitutive protein. After stimulation with 0.2-20.0 u/ml of human recombinant IL-1α there was a dose-dependent, up to 3-fold increase in BM40/SPARC mRNA levels. Cells exposed to phorbol myristate acetate (10^{-6}M) which activates Ca^{2+} dependent protein kinase C and modulates endothelial cell shape induces a similar increase of BM40/SPARC.

These results demonstrate that human microvascular endothelial cells express gene transcripts of BM40/SPARC and upregulate mRNA levels in response to inflammatory and/or PKC-stimulating signals. The Ca^{2+} binding capacity of BM40/SPARC and the central role of Ca^{2+} in modulating endothelial cell shape indicate that BM40/SPARC may be part of the mechanism involved in the IL-1α mediated activation of microvascular endothelial cells.

MURINE KERATINOCYTES EXPRESS INTERLEUKIN-7

Christine Heufler*, Diane Young, Christian Peschel, and Gerold Schuler*, Dpt. of Dermatology* and Internal Medicine, Univ. of Innsbruck, Austria

Interleukin 7 (IL-7) provides a direct proliferative signal to precursors of B (pro-B and pre-B cells) as well as T cells (CD4- CD8- thymocytes) and is expressed by bone marrow stroma, spleen, and thymus. As the dendritic epidermal T cells (DETC) of murine epidermis resemble subsets of CD4-CD8- thymocytes and recent studies by Elbe et al. suggest that fetal epidermis can promote the proliferation of DETC we asked whether murine keratinocytes (K) can express IL-7. We found that subconfluent BALB/c K cultures upon stimulation with LPS or TPA (16 hours) express IL-7 as detected by Northern blot analysis using an IL-7 specific cDNA probe. The PAM 212 K cell line expressed IL-7 even without stimulation. Preliminary experiments suggest that K can release biologically active IL-7. When IL-7 responsive pre-B cells were cocultured with keratinocytes, pre-B cells were viable after 48 hours provided LPS had been added. As a control the pre-B cell line was cultured in medium +/- IL-7 or LPS. We are currently adressing the hypothesis that IL-7 is expressed by murine K under certain conditions (e.g. following UVB irradiation or in fetal epidermis) and causes DETC to proliferate.

CHANGES OF PLASMINOGEN ACTIVATOR (PA) AND PA INHIBITOR (PAI) IN NORMAL HUMAN EPIDERMAL KERATINOCYTE CULTURE. Toshihiko Hibino, Ikuo Segawa and Saiichi Kon. Department of Dermatology, Iwate Medical University School of Medicine, Morioka, Iwate 020, Japan.

In normal epidermis, PAI activity is dominant and PA remains at undetectable level (Hibino et al, FEBS Letters, 1987), while cultured keratinocytes produce and secrete high level of PA. In the present study, we investigated changes of PA and PAI activities in epidermal cell culture. Normal human epidermal keratinocytes (NHEK) were grown in serum free medium (modified MCDB 153) supplemented with bovine pituitary extract (0.4%). PA activities in culture media were mesured every day with two step method using dog plasminogen and synthetic substrate Val-Leu-Lys-pNA. PA activity in the media increased with culture days up to the 9th day when cell became confluent. Approximately 2.4 IU/ml of PA activity was maintained till the 15th day of the culture when spontaneous shedding began. However, PA activity/cell showed its peak activity at the 4th day (4.43 ± 2.07 mIU/ml/cell) and sharply declined. Addition of the purified epidermal PAI into culture media caused a delay to reach cell confluency. Anti-u-PA Fab had a similar effect on keratinocyte culture. Immunohistochemical study using anti-u-PA Fab conjugate showed localization of u-PA from basal to shed cells of the cultured epidermis. On the other hand, PAI activity was not detected in media throught the NHEK culture, although a weak staining for epidermal PAI was shown in denucleated cells. Expression of u-PA from basal to highly differentiated cells and disappearance of PAI activity in cultured epidermis are quite similar to those found in psoriatic epidermis. These data suggest that regulation of PA could have a key role in epidermal differentiation.

THE TRANSCRIPTION OF LORICRIN IS BLOCKED BY RETINOIC ACID Daniel Hohl[1,2,3], Ulrike Lichti[3], Peter Steinert[2] and Dennis Roop[3,4]; Dermatology, University Hospital of Zürich[1], Dermatology Branch[2] and LCCTP[3], N.C.I., N.I.H., Bethesda, Maryland and Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, Texas[4]

We have previously shown that loricrin is a major component of the cornified cell envelope (CE) in mammals and that in contrast to involucrin its expression appears very late in terminal epidermal differentiation in the upper granular layers of normal skin. Submerged and serum-free cultured normal human keratinocytes were grown under various conditions and loricrin mRNA levels were assessed at various time points. As expected, only Ca^{2+} concentrations above 0.1 mM Ca^{2+} were permissive for the expression of loricrin mRNA. Maximal mRNA levels were found at 0.35 mM Ca^{2+} and a critical density appeared to be required for the transcription of loricrin. Retinoic acid (RA) at 10^{-7} to 10^{-9} M completely blocked loricrin transcription when applied simultaneous with increased Ca^{2+}. Furthermore, addition of RA to cultures already exposed to Ca^{2+} resulted in the decrease of loricrin mRNA after 72 hours. So far, no other components of the CE have been shown to be suppressed by retinoic acid. However, similar patterns of expression were reported for filaggrin. Therefore, we compared the mRNA levels of loricrin and the filaggrin precursor and found them to change in parallel in response to the various culture conditions. These results suggest that Ca^{2+}, density and retinoic acid are crucial regulators of loricrin expression in vitro and that the transcriptional control of loricrin and filaggrin expression might be closely coordinated.

ALL-*TRANS*-RETINOIC ACID (RA) SUPPRESSES ORNITHINE DECARBOXYLASE (ODC) ACTIVITY AND POLYAMINE PATHWAY IN CULTURED HUMAN KERATINOCYTES. Noreen J. Hickok, Kathryn Gay, David R. Olsen and Jouni Uitto, Jefferson Medical College, Philadelphia, PA.

RA rapidly down-regulates ODC gene expression in cultured keratinocytes, as determined at mRNA level (*J. Invest. Dermatol.* 94:33-36, 1990). In this study, we examined the consequences of reduced ODC gene expression by determining the ODC activity in cultured keratinocytes incubated with $5x10^{-7}$M RA. The concentrations of the polyamines were also determined by HPLC. Human keratinocytes were cultured under serum-free conditions in KGM (containing 1 μM putrescine) or KGM without putrescine (KGM-P). When incubated in KGM-P, ODC activity was reduced by RA, with maximum inhibition (75%) at 12 h. Assay of putrescine in cultures incubated in KGM-P showed 69 and 80 % reduction at 24 and 48 h, respectively. Spermidine concentration was slightly (30%) reduced at 96 h, while no differences in spermine concentration were noted. As polyamines are obligatory for cell growth, we determined the rate of keratinocyte proliferation by [^{3}H]thymidine incorporation. Preliminary results from cultures incubated in KGM-P suggested a slight inhibition by RA at 48 h. This effect was abrogated by the addition of exogenous putrescine. The results indicate that reduction of ODC gene expression elicited by RA leads to reduced ODC activity and polyamine concentrations, and consequently, to reduced cell proliferation. The results may explain the clinical observations made on patients treated with retinoids for hyperproliferative epidermal disorders.

SPHINGOLIPID SYNTHESIS IN MURINE EPIDERMIS IS REGULATED BY PERMEABILITY BARRIER REQUIREMENTS. Walter M. Holleran, Kenneth R. Feingold, Man Mao-Quiang, Barbara E. Brown and Peter M. Elias. Dermatology and Medicine Services, Veterans Administration Medical Center, and Departments of Dermatology and Medicine, University of California School of Medicine, San Francisco, CA.

Although several lines of indirect evidence point to a role for sphingolipids (SPL) in epidermal barrier function (Grubauer, et al., J. Lipid Res. 30:89-96, 1989), direct evidence for such is lacking. In this study, we first assessed the activity of serine palmitoyl transferase (SPT), the rate-limiting enzyme for SPL synthesis, at various time points (0-24 hrs) after disruption of the barrier with acetone as measured by trans-epidermal water loss. SPT activity was stimulated by acetone treatment, initially at 6 hrs, and returned slowly towards normal after 12 hrs in association with barrier recovery. Moreover, topical application of chloroalanine, a suicide inhibitor of SPT, slowed the recovery of barrier function after acetone treatment, but only at time points greater than 12 hrs. Furthermore, greater than 50% inhibition of SPT activity was observed six hrs following a single application of chloroalanine after barrier disruption. Finally, SPT activity was increased in association with barrier disruption following tape-stripping (22%) and in essential fatty acid deficiency (41%), changes which were inhibited by occlusion. These studies: 1) support a role for sphingolipids in the maintenance of normal barrier; and 2) demonstrate that the regulation of sphingolipid synthesis by barrier requirements differs from the more rapid responses in cholesterol and fatty acid metabolism that occur after barrier disruption.

EFFECT OF EPIDERMAL GROWTH FACTOR (EGF) ON DORSAL SKIN TEMPERATURE IN THE NEWBORN RAT: ANALYSIS BY INFRARED IMAGING SB Hoath, WL Pickens, R Tanaka, MM Donnelly. Dept. of Pediatrics, Univ. of Cincinnati, OH

Exogenous EGF elicits a cascade of predictable events in the newborn rodent ranging from acute changes in skin viscoelasticity to early unfusion of the eyelids. This study was designed to characterize one of the earliest and most sensitive in vivo effects of EGF; i.e., reduction in dorsal skin temp (Growth, Dev. & Aging, 52:77-83, 1988). A total of 112 one-day-old rat pups was examined. Serial dorsal skin temps were recorded from the interscapular integument using a noncontact infrared imaging radiometer. In all experiments, pups were preequilibrated for 1 hour in a C-100 Air-Shields incubator either at nest temp (34°C) or with mild cold stress (30°C). EGF (1-316 ng/g BW) or vehicle was administered and temp measurements performed every 15 min thereafter for 3 hours. At 34°C, 90 min after either vehicle or EGF (316 ng/g BW) skin temps were 36.2 ± 0.2°C vs 35.0 ± 0.3°C ($p<0.01$), respectively. At 30°C, skin temps were 35.6 ± 0.3°C vs 33.3 ± 0.1°C ($p<0.001$). At both ambient temps, the EGF-Rx pups showed recovery to control values by 180 min following Rx. The minimal effective dose was 31.6 ng/g BW at 34°C and 10.0 ng/g BW at 30°C. Pretreatment (60 min) with indomethacin (100 ng/g BW) or ibuprofen (1000 ng/g BW) reduced the maximal EGF response by 45% and 61%, respectively.

Conclusions: 1) EGF elicits an acute, sensitive, dose dependent decrease in interscapular skin temp in the newborn rat; 2) this in vivo effect is potentiated by mild cold stress; 3) pretreatment with indomethacin or ibuprofen partially blocks the EGF effect.

Speculation: EGF may function as a thermoregulatory molecule in the newborn rat.

CHARACTERIZATION OF SERINE-PALMITOYL TRANSFERASE FROM HUMAN NEONATAL KERATINOCYTES AND MURINE EPIDERMIS. W.M. Holleran, M.L. Williams, W.N. Gao, P.M. Elias. Dermatology Service, VAMC & Department of Dermatology, Univ. Calif. School of Medicine, San Francisco, CA.

Sphingolipids (SPL), which comprise ≈25% of human stratum corneum lipids, are thought to mediate epidermal barrier function. Serine-palmitoyl transferase (EC2.3.1.50;SPT) catalyzes the rate-limiting step in SPL synthesis; i.e., synthesis of 3-keto-sphinganine from palmitoyl CoA and serine. We report here the initial isolation and characterization of SPT from cultured human keratinocytes (CHK) and mouse epidermis. In CHK, the specific activity of SPT is 270 ± 20 pmoles/ min/mg microsomal protein (70% of total cellular activity), a level significantly higher than in other tissues. Activity is also high in mouse epidermis (120 - 160 pmoles/min/mg). SPT activity is 30% greater in differentiating than in undifferentiated CHK (grown in 1.2 mM vs. 0.07 mM calcium, resp.). Keratinocyte SPT shows an apparent Km for L-serine of 0.44 mM, with an alkaline pH optimum (8.2 ± 0.4), utilizes palmitoyl-CoA preferentially over other even- or odd-chain, saturated or unsaturated fatty-acids. Finally, the SPT inhibitors, L-cycloserine and β-chloro-L-alanine, are potent inhibitors of SPT in CHK (IC50's of ≈2.5 and 25 uM, respectively). In summary, we have: 1) described the first isolation and characterization of SPT from CHK and epidermis; 2) found abundant SPT activity in CHK and epidermis, consistent with the skin's ability to generate large quantities of SPL; 3) noted increased SPT activity in keratinocytes cultured under differentiation-favoring conditions.

EXPRESSION OF HEPARAN SULFATE PROTEOGLYCAN AND LAMININ IN DEVELOPING HUMAN FETAL SKIN BASEMENT MEMBRANE: DIFFERENCES IN ULTRASTRUCTURAL LOCALIZATION ARE DEPENDENT ON DEVELOPMENTAL AGE. Yuji Horiguchi, Jo-David Fine, John R. Couchman, and Eva Engvall. Departments of Dermatology and Cell Biology & Anatomy, Univ. of Alabama School of Medicine, Birmingham, AL, and La Jolla Cancer Research Foundation, La Jolla, CA.

Human skin basement membrane (BM) zone is an antigenically complex structure composed of a variety of collagenous and non-collagenous proteins, glycoproteins, and proteoglycans. Although it is known that specific antigens and associated structures (i.e., hemidesmosomes; anchoring fibrils) develop in a timely sequence during embryogenesis, no data exists as to the ultrastructural local-ization of specific antigens as a function of gestational age. With this in mind, we have examined a series of human fetal skin specimens (gestational age range = 54-142 days) by immunoelectron microscopy via immunoperoxidase technique using 7 well characterized monoclonal antibodies to human laminin (A & B chains) and heparan sulfate proteoglycan core protein (HSPG). At 54 gestational days, corresponding to the earliest detectable lamina densa (LD) in human fetal skin, each antigen was diffusely present along a rather amorphous DEJ. By 76 days, however, anti-laminin A chain antibody bound primarily to the lamina lucida (LL), whereas anti-laminin B chain bound rather exclusively to the LD. Similarly, HSPG was detectable primarily within and beneath the LD. With advancing gestational age, these differences became more defined, mirroring findings in neonatal foreskin (i.e., by 142 days, lesser amounts of HSPG could also be detected adjacent to hemidesmosomes, within the LL, and even decorating upper dermal collagen fibers). We conclude that the distribution of specific DEJ components varies at different developmental ages in human fetal skin, related in part to the appearance of specific BM-associated structures.

SELECTIVE EXPRESSION OF VLA INTEGRINS IN NORMAL HUMAN NERVE AND IN CUTANEOUS NEUROFIBROMAS. Li Li Hsiao, Juha Peltonen, Sirkku Jaakkola, and Jouni Uitto. Thomas Jefferson University, Philadelphia, PA

The distribution of VLA integrins in human peripheral nerve and in cutaneous neurofibromas was studied by immunostaining for β1, α2, α3, and α5 subunits. In human nerve, the expression of β1 subunit was detected on Schwann cells, perineurial cells, and epineurial blood vessel walls. A distinct difference between developing (fetal) and mature (adult) nerve was seen. In an adult nerve, only a subpopulation of Schwann cells showed an intense staining reaction; these cells ensheathed a group of small-diameter axons, as detected by double immunofluorescence with an anti-neurofilament protein antibody. In contrast, in a fetal nerve, which contains only small-diameter axons, all Schwann cells expressed the β1 epitopes apparently at the same level. The α2 subunit was expressed only on endoneurial Schwann cells both in fetal and adult nerve, while the α3 epitopes were expressed exclusively in the adult tissue and were primarily present on perineurial cells and epineurial vessel walls. The α5 epitopes were expressed only on the innermost compartment of perineurium of an adult nerve. The tumor cells within cutaneous neurofibromas expressed α2 and α3 subunits, while α5 epitopes were absent. Cell cultures established from human fetal nerve revealed expression of the α2 and α5 epitopes both on Schwann cells, perineurial cells and fibroblasts, while only Schwann cells contained the α3 epitopes. The results suggest a role for VLA integrins in cell-cell and cell-matrix interactions during nerve development and homeostasis.

The possible role of protein kinase C in the regulation of melanocyte growth and pigmentation.

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The role of protein kinase c in melanocyte growth and tyrosinase activity was examined using H-7 [1-(5-Isoquinolinesulfonyl)-2-methyl piperazine dihydrochloride] :potent and selective protein kinase c inhibitor, and (OAG-1-oleoyl-2-Acetyl glycerol): synthetic glycerol. In normal epidermis in vivo melanocytes undergo little replication. TPA (12-o-tertradecanoyl-phorbol-13-acetate), however, supports the proliferation of normal human melanocytes in culture. Withdrawal of TPA from the culture medium leads to reduction in the dendritic processes, dilatation of cytoplasm, inhibition of growth and death after 5-7 days. TPA is known to be a protent promoter and has been shown to activate directly protein kinase c (Ca-activated, phospholipid-dependent protein kinase). The growth of melanocyte was inhibited by 60% with addition of H7 (20 μ M). OAG (20 μ g/ml) did not support the growth, leading to death of melanocyte. The cytotoxic effects of OAG were reduced by the addition of H7. Tyrosinase activity was not altered by H7. These data indicated that the growth and pigmentation of melanocyte may not be regulated by the direct activation of protein kinase c.

EPIDERMOLYSIS BULLOSA: EVIDENCE FOR LINKAGE TO GENETIC MARKERS ON CHROMOSOME 1 IN A FAMILY WITH AUTOSOMAL DOMINANT SIMPLEX FORM. MM Humphries, D Sheils, M Lawler, GJ Farrar, P McWilliam, P Kenno, DG Bradley, EM Sharp, EF Gaffney, M Young, J Uitto, and P Humphries. Departments of Genetics, Histopathology and Occupational Therapy, Trinity College, Dublin, Ireland; and Departments of Dermatology, Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Philadelphia, PA.

A 3-generation pedigree of Irish origin, including 14 affected individuals and displaying an autosomal dominant simplex form of epidermolysis bullosa (EB) of the Koebner variety, was examined. Electron microscopy of the skin revealed focal basal cell degeneration and cleavage between basal cells and immediately overlying suprabasal keratinocytes. DNA was isolated from the peripheral blood leukocytes and analysed by a combination of Southern blotting and polymerase chain reaction for linkage with a set of polymorphic markers derived from the long arm of chromosome 1. Two point analysis revealed positive lod scores for four of these markers, AT3 (Z=2.107, θ=0), ApoA2 (Z=1.939, θ=0.15), DIS13 (Z=1.026, θ=0.15) and DIS65 (Z=0.329. θ=0.15). Multi-locus analysis, incorporating the markers DIS19, DIS16, DIS13, ApoA2, AT3 and DIS65, resulted in a lod score of 2.64 maximizing over the region between DIS13 and DIS65. These data strongly support previous tentative indications of a linkage between autosomal dominant simplex EB and genetic markers on the long arm of chromosome 1.

RAPID QUANTITATION OF CROSS-LINKED PROTEIN AS AN ALTERNATIVE TO COUNTING CORNIFIED ENVELOPES. Lynne Hough-Monroe and Leonard M. Milstone. VA Hospital and Yale Medical School, New Haven, CT

The cornified envelope, consisting of covalently cross-linked protein, is a key feature of keratinocyte differentiation. Enumeration of cornified envelopes is widely used to assess keratinocyte differentiation. In order to avoid the tedium, subjectivity and notorious inconsistencies of the usual visual assays for cornified envelopes, we developed a rapid, objective, sensitive, quantitative and reproducible assay that measures total cross-linked protein in keratinocytes. The method is based on a) resistance of cross-linked protein to solubilization in boiling SDS/βME, b) trapping of cross-linked protein on filter paper, c) binding of Coomassie Blue (G-250) to protein and d) scanning laser densitometry of stained spots. Keratinocytes boiled in 2%SDS-1%βME are applied to regenerated cellulose sheets (S&S #RC60) in the wells of a manifold (S&S Minifold I) and washed 5 times with SDS/βME to remove soluble protein. Entire sheets are then stained and washed as described by Bramhall (Anal Biochem 31:146, 1969) and scanned in a laser densitometer (Molecular Dynamics). The assay detects protein from $\geq 10^4$ envelopes. In a given population of cells, there is excellent correlation between the cross-linked protein and the number of cornified envelopes. The amount of cross-linked protein correlates with keratinocyte differentiation. The assay does not exclusively measure protein in cornified envelopes nor does it distinguish between populations containing few cells with much envelope protein or many cells with little envelope protein.

TRANSCRIPTIONAL ACTIVATION OF C-FOS GENE NEGATIVELY REGULATES PROLIFERATION OF HUMAN MELANOCYTES AS A RESPONSE TO PHORBOL ESTER APPLICATION. Zaheed Husain and Michael M. Wick. Dana-Farber Cancer Institute and Dept. of Dermatology, Harvard Medical School, Boston, MA.

Normal human melanocytes isolated from neonatal foreskins were cultured in defined media in the presence of the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA). The presence of TPA supports rapid proliferation of cells under these conditions and is required for the successful cultivation of normal melanocytes *in vitro*. Withdrawal of TPA from the media causes restriction in the growth of cells along with alteration in morpholgy and increased melanization. Readdition of TPA reverses these effects and restores cells to their proliferative state. Study of mRNA levels of several early response genes under these conditions shows that the basal level of expression of the c-fos gene, which is low in the proliferative state, is rapidly induced by withdrawal of TPA from the media. Addition of TPA back to the media after 48 hours decreases the rate of transcription of c-fos while the state of expression of several other genes remains unchanged. Transient c-fos transcription may have a role in negatively regulating proliferation of normal human melanocytes in culture.

TRANSCRIPTION ACTIVATING FUNCTIONS OF THE RAT ESTROGEN RECEPTOR. Sumihisa Imakado, Yasumasa Ishibashi, Satoshi Koike*, Shigeru Kondo**, and Masami Muramatsu**, Department of Dermatology and **Department of Biochemistry, Faculty of Medicine, Tokyo Univ., Tokyo and *the Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

The estrogen receptor (ER) belongs to the nuclear receptor family, a class of DNA binding proteins that act as ligand-activated enhancer factors and includes the steroid/thyroid hormone receptors, vitamin D3 and retinoic acid receptors. We isolated the rat ER cDNA clone from a cDNA library of the rat uterus(1). Its transient expression in transfected COS7 cells resulted in the synthesis of a protein which bound estradiol with the same affinity as the endogenous rat uterus ER. To identify the transcription activating domains of the rat ER, we made a number of deletion mutants and transfected the mutant plasmids into COS 7 cells together with an estrogen-responsive reporter plasmid ERE-tk(200)-CAT, which contains the ERE of Xenopus vitellogenin A2 gene. We now show clearly that the rat ER has two transcription activating domains, one is in the N-terminal and the other is in the C-terminal region.

(1) Koike,S.,et al.(1987) Nucleic Acid Res.,15,2499-2513.

CHARACTERISTICS OF PROTEIN KINASE C/PHORBOL ESTER RECEPTORS IN PROLIFERATING AND DIFFERENTIATED HUMAN KERATINOCYTES. R.R. Isseroff, and J.L. Gross, University of California, Davis, CA and DuPont & Co., Wilmington, DE.

Activation of protien kinase C (PKC) is a mechanism by which extracellular signals are transduced and control cell growth and differentiation. To understand the role PKC plays in the control of keratinocyte growth, we examined the characteristics of PKC in proliferating (subconfluent, SC), and differentiated (post-confluent, PC), human neonatal keratinocyte cultures by quantiting the binding of the phorbol ester, ^{3}H phorbol 12,13-dibutyrate (^{3}H-PDBU), to its receptor, PKC. When binding to intact cells was examined, Scatchard analysis of the binding isotherms fitted for the model of a single (major) class of binding sites, revealed significant differences between the Kd of the SC cells (47.4nM) as compared to the PC cells (20.5nM), $p<.05$. To examine the subcellular localization of PKC, SC or PC cultures were lysed and fractionated and ^{3}H-PDBU binding to cytoplasmic and particulate fractions was determined:

	Kd(nM)		BMax(pMol/mg protein)	
	Subconf	Postconf	Subconf	Postconf
Cytoplasm	4.5	2.2	0.39	0.33
Particulate	1.8	5.1	0.52	0.54

The relative distribution of receptors (80-90% cytoplasm, 10-20% particulate) was not significantly different in the two cell populations. This subcellular distribution could be altered, however, by 5 min. pretreatment of the cells with PDBU, with a redistribution to 40% binding in the cytoplasm and 60% particulate. The observed differences in the affinities of the phorbol receptor in the proliferative and differentiated cell populations indicate that changes in the relative activity of PKC may play a role in keratinocyte differentiation.

SELECTIVE RECOVERY OF DIMINISHED CUTANEOUS BARRIER FUNCTION IN ESSENTIAL FATTY ACID DEFICIENT RAT BY SYNTHETIC O-ACYLCERAMIDE WITH LINOLEIC ACID. Genji Imokawa, Minehiro Okuda, Yukihiro Ohasi and Akira Kawamata, Kao Corporation, Biological Science Laboratories, Tochigi, Japan.

Sphingolipids play an important role in cutaneous permeability barrier and specifically O-acylceramide with linoleic acid has been considered its main functional component. In attempt to study the significance of O-acylceramide with linoleic acid in controlling the barrier function, we synthesized pseudo O-acylceramides with different O-acyl properties and examined the potential to restore the diminished barrier function in essential fatty acid deficient rat by their topical applications. Barrier function was assessed by measuring both transepidermal water loss(TEWL) and penetration rate of ^{14}C-salicylate through the separated skin. Daily applications of synthetic O-acylceramide with linoleic acid solubilized at 10% in squalane base caused a significant decrease of TEWL, accompanied by a marked suppression in the penetration rate as compared with squalane base, whereas other O-acylceramides with oleic acid or saturated alkyl chains, or ordinary ceramides did not exhibit any significant recovery in either TEWL or the penetration rate. To rule out the possibility that linoleic acid released enzymatically from applied O-acylceramide serves the observed recovery, ether-linked linoleic acid-containing ceramide was also applied in the same manner. It also induced a significant and similar increase in the barrier function. These findings directly indicate the significant role of O-acylceramide with linoleic acid in cutaneous barrier function.

TISSUE FACTOR EXPRESSION AND REGULATION IN HUMAN KERATINOCYTES AND EPIDERMIS. P. Itin, J. Wilcox, R. Coffey, M. Pittelkow. Depts. of Dermatology, Cardiovascular Research and Medicine, Mayo Clinic, Rochester, MN., Genentech, San Francisco, CA. and Vanderbilt Univ, Nashville, TN.

Tissue factor (TF) is a membrane-bound glycoprotein that functions in the initiation of blood coagulation. TF is sequestered in selective tissues and activates clotting when vascular integrity is interrupted. TF is one of a set of immediate-early gene products that may mediate the cellular growth response. We investigated the expression of TF in epidermis and cultured human keratinocytes (HK) and characterized modulation of TF by growth regulators. Antibodies to TF localize the protein to the upper stratified and granular layers of uninjured epidermis. Confluent cultures of HK grown in serum-free medium express TF protein as detected by ELISA. Bioactivity of TF is detected by a chromogenic assay for coagulation factor activation. Confluent HK cultures deprived of exogenous growth factors for 48 hr contain approximately 20 ng TF/10^{6} cells. Addition of EGF or serum stimulate TF protein production within 6 hr; maximum accumulation occurs by 12 hr and decreases thereafter. The phorbol ester, TPA, does not significantly enhance TF production but induces a marked decrease in TF protein after 12-24 h of treatment. TF mRNA expression by HK is markedly induced (4-5 fold) within 2 hr of treatment by EGF, TGF-α or serum. In summary, TF protein is present in the differentiated layers of normal epidermis and is expressed in cultured HK. Growth factors and serum significantly enhance TF mRNA and protein expression by HK. TF expression appears to be linked to epidermal differentiation *in vivo* and TF mRNA and protein are modulated by the growth state and growth factors *in vitro*. Besides its role in coagulation, TF may function in regulation of epidermal proliferation and/or differentiation.

DIFFERENCES IN REGIONAL EXPRESSION OF PEMPHIGUS FOLIACEUS, ERYTHEMATOSUS AND VULGARIS ANTIGENS IN HUMAN SKIN. D. Ioannides, P. Hytiroglou, R. Phelps and J-C Bystryn, Dept of Dermatology, NYU School of Medicine, and Dept of Pathology, Mount Sinai School of Medicine, New York, NY.

The possibility that the distribution of skin lesions in pemphigus foliaceus (PF) and erythematosus (PE) is due to variations in the antigenic properties of skin was studied. The expression of PF, PE and PV antigens in skin specimens obtained from 15 different locations in each of 3 individuals was evaluated by indirect immunofluorescence using sera obtained from 2 pts each with PF, PE or PV and high titers of intercellular antibodies (titer:640 vs guinea pig and monkey esophagus). Antigen concentration was expressed as the highest dilution of serum (end-point titer) that stained the skin specimen. The expression of PF and PE antigens was highest on the upper back, upper chest and xiphoid (average end-point titers of 871 and 262 respectively), and lowest on buccal mucosa, lower abdomen, lower back and scalp (average end-point titers of 210 and 22 respectively). This contrast to the distribution of PV antigen which was highest on buccal mucosa and scalp.

These results indicate that there are regional differences in the expression of PF and PE antigens, that the distribution of these antigens differs from that of PV antigens, and suggest that these differences may account in part for the differences in distribution of skin lesions in different forms of pemphigus.

ICHTHYOTIC MOUSE - STRUCTURAL AND BIOCHEMICAL ANALYSIS OF THE HAIR DEFECT. Peter H. Itin, John P. Sundberg*, Robert W. Dunstan* and Mark R. Pittelkow, Department of Dermatology, Mayo Clinic, Rochester, MN and *Jackson Laboratory, Bar Harbor, ME.

Hair of the homozygous ichthyotic (ic/ic) mouse is fine, short and sparse with curls. Hair shaft thickness is irregular. Cuticles are absent or decreased in size. The medulla is irregular along the longitudinal axis. We determined that specific structural or biochemical abnormalities account for the characteristic hair defect in this mutant strain. Light microscopy demonstrated an irregular and altered pattern of dark and light banding that was optically enhanced by using polarizing microscopy. Scanning electron microscopy revealed severe cuticular degeneration. X-ray microanalysis of hair sulfur detected lower sulfur content in the ic/ic mouse hair (80% of control) although individual hairs showed variable deficiency. Examination along the longitudinal axis of individual normal and ichthyotic hairs also showed variable sulfur content. Amino acid analysis revealed no marked difference from control specimens of hair. These investigations demonstrate structural and select biochemical changes in the ic mouse hair. This autosomal recessive defect localized to mouse chromosome 1, may affect the synthesis of filamentous or matrix proteins of hair. Further investigations are necessary to delineate the molecular defect in hair of the ic mouse and determine if the ic mouse may be useful as an animal model for human hair disease.

COMPARATIVE STUDY OF IN SITU DEMONSTRATION OF PROLIFERATING CELLS IN SKIN TUMORS USING MONOCLONAL ANTIBODIES.

Kaoru Ito, Masaya Tezuka, Nobue Yoshida, Masaaki Ito and Yoshio Sato, Department of Dermatology, Niigata University School of Medicine, Niigata, Japan.

In order to detect proliferating cells in skin tumors in situ, immunohistological study was performed using anti-proliferating cell (Ki-67), anti-DNA polymerase α and anti-bromodeoxyuridine (BrdU) monoclonal antibodies (MoAbs).

Tissues of basal cell epithelioma (BCE) (n=4), Bowen's disease (n=4), malignant melanoma (MM) (n=3) and squamous cell carcinoma (SCC) (n=3) were examined. Ki-67 was stained by immunofluorescence and avidin-biotin-peroxidase complex (ABC) immunoperoxidase methods. Anti-DNA polymerase α MoAb was used with peroxidase-anti-peroxidase (PAP) method. BrdU incorporated into proliferating cell was detected with anti-BrdU MoAb by ABC method. Cells with positive nuclei were detected with these methods.

Staining with these methods gave similar results. In BCE, positive cells were scattered in the periphery and the center of tumor nests. In Bowen's disease, a large number of positive cells were observed not only in the basal layer but also in the upper portion of the tumors. In SCC and MM, moderate number of positive cells were detected in the tumors. Among these methods, Ki-67 sometimes gave cytoplasmic staining which made the evaluation uneasy. Therefore detection using anti-BrdU and anti-DNA polymerase α MoAbs seemed to be more reliable in detecting proliferating cells in situ.

IMMUNOLOCALIZATION OF HUMAN THYROID HORMONE RECEPTOR IN INTACT SKIN AS WELL AS IN CULTURED KERATINOCYTES AND FIBROBLASTS. Anthony M. Janiga, Enrico Macchia, Maria-Elena Fisfalen, Aimin Tang, Leslie J. DeGroot, Keyoumars Soltani, University of Chicago, Chicago, IL.

Cutaneous changes are commonly observed in thyroid disease, and it is known that thyroid hormone plays a pivotal role in normal cutaneous function. The presence and localization of human thyroid hormone receptors (hTRs) in the skin has not yet been demonstrated. Polyclonal antibodies were developed by immunizing rabbits with synthetic peptides representing antigenic epitopes in the molecule including amino acid residues 31-47 and 62-81 of hTR and 17-33 and 144-162 of hTR . The antibody sensitivity was assessed by ELISA ($1:10^4$-$1:10^5$) and the specificity was confirmed by preabsorption with homologous peptides and immunoprecipitation using ^{125}I-T_3 labeled rat liver nuclear extract. Each antibody caused a partial shift of the ^{125}I-T_3 receptor complex to a higher molecular weight form. Preincubation of the antisera with their corresponding peptides eliminated this shift. The receptor localization was studied by immunohistochemical (ABC kit) and indirect immunoflourescence (IIF) techniques. Human skin sections and cultured human keratinocytes and fibroblasts were fixed in cold methanol. Immunolocalization of the hTR was demonstrated by nuclear staining in essentially all cultured keratinocytes and fibroblasts. However in intact human skin sections nuclear staining was limited to dermal fibroblasts and to basal and mid-level keratinocytes. Thus for the first time the presence of hTRs has been demonstrated in intact human skin.

COMPUTER-STEREOGRAPHIC AND ELECTRON MICROSCOPIC STUDY OF MONILETHRIX. Masaaki Ito, Ken Hashimoto*, Kaoru Katsuumi, Kaoru Ito, Tatsuya Takenouchi, and Yoshio Sato, Dept. of Dermatology, Niigata Univ. School of Medicine, Japan and *Wayne State Univ. School of Medicine, Detroit, Michigan.

The plucked hairs and biopsied hair tissues of the scalp were obtained from two patients with monilethrix. By SEM, the plucked hairs showed a typical moniliform feature. By computer-stereography, reconstructed three-dimensional models of hair tissues showed that the diameter of hair shaft was partially reduced in the keratogenous zone and that the reduction was severe in the hair cortex but mild in the inner root sheath. By TEM, a marked degeneration of hair matrix cells and invaginations of the hair cuticle cells into the cortex in the suprabulbar portion were found. In some hair tissues, the hair bulbs showed no degeneration, but a degeneration of cortical cells and invaginations of hair cuticle were seen in the developing zone of the cortex. This suggests that not every hair matrix is damaged in a synchronized fashion but individual hair is affected independently. In all the hair tissues examined, cytoplasmic vacuolations in the various layers and abnormal formation of tonofibrils in the cortex were often seen. When an inherent cell abnormality occurs severely, the cortical cells are particularly affected in the hair matrix; this seems to result in a decrease in number of cortical cells and thinning of the hair shaft.

EFFECTS OF LOCAL UV IRRADIATION ON IMMUNITY TO BCG INFECTION IN MICE. Amminikutty Jeevan and Margaret L. Kripke, Department of Immunology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Recently, we demonstrated that exposure of mice to a high dose of UV radiation caused systemic suppression of the delayed type hypersensitivity (DTH) response to BCG in mice and impaired their ability to eliminate viable bacteria from the lymphoid organs. In this study, we wished to determine whether mice given low doses of UV radiation would exhibit similar impairments in host resistance to BCG injected into the site of irradiation. BALB/c and C3H/HeNCr mice were exposed on dorsal skin to UV radiation from a bank of 6 FS40 sunlamps for 1 min on 4 consecutive days. The total dose of UVB (280-320 nm) radiation delivered was 1.4 kJ/m^2. On the last day of UV treatment, mice were infected with Mycobacterium bovis BCG by i.d. injection of $5x10^6$ live BCG (Tice strain) into the irradiated skin. At various intervals, groups of mice were tested for DTH by injection of 50 µl PPD into the hind footpads and measuring the footpad swelling 24 h later. The course of BCG infection was followed by counting the number of viable bacteria in the draining lymph nodes and spleen. Local UV irradiation had no effect on the induction of the DTH response to PPD in either strain of mice; in fact, the response was enhanced in UV-irradiated mice. However, the number of viable bacteria was increased significantly in the lymphoid organs of the UV-irradiated mice. These results indicate that the DTH response to BCG is not impaired by local UV irradiation, but that this treatment interferes with clearance of the bacteria, indicating a dissociation between the development of DTH and resistance to infection.

TRAUMA INDUCED FROM SIMULTANEOUS SUBTHRESHOLD UV-B AND IR IRRADIATION. S. Jadlos, T. Mammone, N. Muizzuddin, K. Marenus, D. Maes and W. Smith, Estee Lauder R&D, Melville, NY.

We have found that simultaneous administration of subthreshold doses of UV-B and IR leads to an inflammatory response both in-vitro and in-vivo. In-vitro, phospholipase mediated release of arachidonic acid (AA) in keratinocytes is indicative of cellular damage and predictive of inflammation in-vivo. Both UV-B and IR alone are capable of stimulating AA release at doses of approximately 400/mJ/sq cm and 50 J/sq cm respectively. When subthreshold doses of UV-B (100 mJ/sq cm) and IR (25 J/sq cm) were administered simultaneously, the release of AA was found to be significantly higher than for either UV-B or IR alone. These data are consistent with in-vivo results reported previously and clinical data obtained in our lab. When suberythemal doses of UV-B and IR were administered simultaneously significant increases in capillary blood flow, erythema and T.E.W.L. were observed. These data indicate the importance of considering possible additive effects from simultaneous irradiation at several wavelengths which occur during normal sun exposure.

DETECTION OF HERPES SIMPLEX VIRUS DNA IN LESIONS OF RECURRENT APHTHOUS STOMATITIS USING THE POLYMERASE CHAIN REACTION. JD Jester, SS Stockert, JC Huff, SL Brice, Dept of Dermatology, Univ of Colorado School of Medicine, Denver, CO.

Recurrent aphthous stomatitis (RAS) is a common disease of the oral mucosa which may be associated with significant morbidity. The pathogenesis remains unclear although a role for herpes simplex virus (HSV) has been proposed. Previous attempts to demonstrate HSV in RAS lesions using culture and immunofluorescence techniques have been unsuccessful. However, the lesions examined were advanced so that the virus may no longer have been detectable. In addition, no studies to detect viral nucleic acids have been reported. The purpose of this project was to examine early lesions of RAS for the presence of HSV DNA using the polymerase chain reaction (PCR). Eleven patients with RAS were studied. Sera were examined for HSV antibodies by ELISA. Biopsies of RAS lesions less than 2 days old were obtained from each patient. At that same time, biopsies of noninvolved mucosa were obtained from 5. DNA was extracted and examined for HSV using the PCR with HSV specific primers. Six patients were HSV antibody positive and 5 were negative. HSV DNA was detected in RAS lesions from 4/6 antibody positive patients. No HSV was detected in lesions from the 5 antibody negative patients. None of the 5 biopsies of noninvolved mucosa was positive for HSV including 2 from patients with positive lesional biopsies. These results suggest that HSV may be involved in the pathogenesis of RAS in patients who are HSV antibody positive.

AN ENHANCER CONTROLS HUMAN K#14 KERATIN GENE EXPRESSION. C.-K. Jiang, M. Tomic, H. Epstein, I.M. Freedberg, and M. Blumenberg, Department of Dermatology, New York University Medical Center, New York, NY.

We have shown previously that the promoter of the K#14, basal cell-specific, keratin gene confers epithelial-specific expression. We now show that the same 300 base pair (bp) segment has enhancer-like properties: it can increase transcription from enhancer-less promoters several hundred fold. This increase is independent of the orientation and position of the 300 bp segment. The enhancement is epithelial specific since it does not occur in non-epithelial cells, such as fibroblasts, but does occur in HeLa cells and in primary cultures of epithelial cells.

Deletions of the 300 bp segment were prepared and their function as an enhancer and as a promoter determined. The segment containing the fragment from -240 to -140 bps, relative to the start codon, contained enhancer activity. Constructs extending to positions -270 and -230 were fully functional in epithelial cells. Constructs extending to -190 and -160 were not, which identifies essential functional elements in the DNA segment between -230 and -190. All functional constructs were epithelial specific which indicates that they depend on epithelial-specific, trans-acting proteins for their activity. We proved this directly by showing that the 300 bp segment does not function as a silencer in non-epithelial cells.

These results point to the specific upstream sequences which control expression of the K#14 keratin gene in human epidermis.

DOSE DEPENDENT INDUCTION OF EFFECTOR AND SUPPRESSOR CELLS OF CONTACT HYPERSENSITIVITY IN NORMAL AND UVR-EXPOSED MICE. Byoung-Deuk Jun and Lee K. Roberts. Chonbuk National Univ Med Sch, Chonju, Korea; and Schering-Plough, Memphis, Tennessee.

Ultraviolet radiation (UVR) causes suppression of contact hypersensitivity (CH) responses. Further, UVR-induced suppression of CH is mediated, in part, by antigen-specific suppressor T-cells (Ts cells). The purpose of this study was to characterize CH effector and Ts cell development in the lymphoid organs of normal mice and determine how UVR exposure effects this process. Normal and UVR exposed mice were sensitized with a high dose of 0.5% DNFB (25 ul, abdomen; 10 ul, ears and feet). After 5 days peripheral lymph nodes (LN) and spleen cells were transferred to syngeneic recipients. They were challenged with 0.1% DNFB on the right ear and after 24 hrs the ear swelling response was measured. CH effector cells were observed in LN and spleen of both normal and UVR-exposed donors. When recipients of these same cells were sensitized after transfer and challenged 5 days later Ts cell activity was observed in the LN and spleen of both normal and UVR-exposed donors. Using low dose 0.5% DNFB sensitization (25 ul, abdomen only) effector cells were transferred with LN and spleen from normal and UVR-exposed donors, however, Ts cells only developed in the LN, and not the spleen, of normal mice. In contrast, LN and spleen from UVR-exposed mice possessed Ts cells. These results indicate that normal immune responses to high dose DNFB induce both effector and Ts cell responses, that low doses of DNFB result in Ts cell development only in the LN, and UVR shifts the dose response curve to DNFB such that low doses of DNFB induce Ts cells in spleen and LN.

AUTOCRINE STIMULATION BY TGF-α IS MORE ACTIVE THAN PARACRINE: TUMORIGENIC TRANSFORMATION OF NIH 3T3 CELLS. William D. Ju, Thierry J. Velu, William C. Vass, and Douglas R. Lowy, Laboratory of Cellular Oncology, National Institutes of Health, Bethesda, MD.

Many highly malignant tumors co-express growth factors and their receptors. Such an autocrine loop occurs commonly in epithelial tumors via the co-expression of the EGF receptor (EGFR) and transforming growth factor-α (TGF-α), its ligand. While one potential advantage for growth stimulation by autocrine circuits is that the tumor cell need not rely on other cells to produce the ligand (paracrine stimulation), we have here tested the as yet unproven speculation that at least in some instances autocrine stimulation may be more potent than paracrine stimulation. To examine this possibility, a cDNA sequence coding for the full length human TGF-α precursor protein was subcloned into a retroviral vector. The vector was then introduced into and expressed in NIH 3T3 cells, which have low numbers of endogenous mouse EGFRs. Control cells not expressing the gene were not transformed by paracrine treatment with concentrations of exogenous human TGF-α that fully saturate and activate the endogenous EGFRs. In contrast, cells that produced their own human TGF-α became morphologically transformed; cells in the same tissue culture dish that did not express the gene were not transformed and exhibited density dependent growth inhibition, indicating that the transformation was due to a direct autocrine effect of TGF-α. When inoculated into nude mice, the TGF-α expressing cells rapidly gave rise to tumors that grew progressively, while control cells did not form tumors. We conclude that autocrine co-expression of TGF-α and EGFRs, as found in numerous cancers, may contribute directly to tumorigenicity. Moreover, these observations provide strong experimental support for the hypothesis that autocrine stimulation of cells can be more potent than paracrine stimulation.

CHARACTERIZATION OF THE IGA ANTIBASEMENT MEMBRANE ANTIBODY FROM LINEAR IGA BULLOUS DERMATOSIS SERA. DP Kadunce, T Taylor, LJ Meyer, and JJ Zone, Division of Dermatology, University of Utah, Salt Lake City, Utah.

Linear IgA bullous dermatosis (LABD) is a rare papulovesicular disorder characterized by the deposition of IgA in a linear pattern along the basement membrane zone (BMZ). This antibody is directed against a 97 kD component of the BMZ. We characterized the circulating IgA anti-BMZ antibody in LABD, as to molecular size, IgA subclass, and presence of secretory component. Antibody reactive against the 97 kD band of dermal extract was purified by elution from nitrocellulose. These immunoaffinity purified antibodies from 3 patients with lamina lucida type LABD, when applied to non-reduced 4% PAGE and immunoblotted versus anti-IgA, anti-IgA_1, anti-IgA_2, and anti-secretory piece, revealed: 1) a band at 155 kD with anti-IgA, anti-IgA_1 and anti-IgA_2; 2) a band at 320 kD with anti-IgA and anti-IgA_1; and 3) a weak band at 390 kD with anti-IgA, anti-IgA_1, and anti-secretory component. Further studies with reduced PAGE systems demonstrated a 70 kD band when stained for secretory piece. The above results were present in all three sera.

From this we conclude that in lamina lucida type LABD: 1) the IgA deposits contain predominantly IgA_1 and smaller amounts of IgA_2 and secretory piece; 2) that the IgA_1 is monomeric and dimeric; and 3) that the IgA_2 is predominantly monomeric. This strongly suggests that the IgA antibody which binds to the lamina lucida in LABD represents a polyclonal rather than a monoclonal immune response.

NAD(P)H:QUINONE REDUCTASE: SAME ENZYMATIC FORM PRESENT IN MURINE LIVER AND SKIN, AND HUMAN KERATINOCYTES. F.Jugert and H.Merk, Department of Dermatology, University of Köln, West-Germany

NAD(P)H:quinone reductase (EC 1.6.99.2) belongs to the aromatic hydrocarbon responsive [Ah] battery. This gene battery includes Cyp 1a1 (Cytochrome P_1450), Cyp 1a2 (cytochrome P_3450), and Nmo-1 (NAD(P)H:quinone reductase (2). In skin cytochrome P_1450 dependent activity is about 1-5% compared to its activity in the liver, whereas NAD(P)H:quinone reductase has the same activity in skin and liver (1), supporting the concept that at least 2 different mechanisms operate in Nmo-1 gene activation. In order to study whether there are the same NAD(P)H:quinone reductase activities in liver and skin of mice and whether the cutaneous human NAD(P)H:quinone-reductase is similar to the murine one we performed an inhibition study with known inhibitors of different quinone reductases (3). - NQR was determined in the cytoplasma of murine skin, liver, and human keratinocytes using 2,6-dichlorophenol-indophenol (DCPIP) as substrate. The enzyme activity was expressed as nmol DCPIP reduced/min/mg protein. In order to characterize this enzyme, induction by polycyclic hydrocarbones and inhibition with several known inhibitors of dihydrodiol dehydrogenase, aldo-keto and carbonyl reductase activities were determined. - The basal NQR-activity was similar in murine skin and liver, however the induction with polycyclic hydrocarbons was stronger in the liver. There was a similar pattern of inhibition of the basal and induced activity in all tissues so far investigated. Pyrazole, progesterone and phenobarbital did not inhibit, however rutin and indomethacin inhibited dose dependently. The most potent inhibitor was dicoumarol. These findings suggest that the same enzymatic form is present in liver and skin, and in murine skin and human keratinocytes.

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2.D.D.Peterson et al., PNAS 86 (1989) 6699-6703
3.K.Post et al., 11th European Workshop on Drug Metabolism (A)., 1988, p. 1.46

HUMAN ELASTIN GENE: DELINEATION OF FUNCTIONAL PROMOTER AND REGULATORY cis-ELEMENTS IN THE 5'-FLANKING REGION. Veli-Matti Kähäri, Michael J. Fazio, Yue Que Chen, Biagio Saitta, Muhamad Bashir, Joel Rosenbloom, and Jouni Uitto, Departments of Dermatology, Biochemistry and Molecular Biology, Jefferson Medical College, and Center for Oral Health Research, University of Pennsylvania, Philadelphia, PA

Analysis of nucleotide sequences in the 5' flanking region of the human elastin gene has revealed several unusual features. We have developed several elastin promoter region/CAT reporter gene constructs by cloning elastin gene 5' flanking DNA into a promoterless Bluescript/CAT construct (pBSOCAT). The spectrum of inserts, spanning from -2,260 to +2, was shown to contain several SP-1 and AP2 binding sites, as well as putative glucocorticoid, cAMP and TPA responsive elements. Assay of promoter activity in transient transfections of rat aortic smooth muscle cells, human skin fibroblasts, HeLa cells or mouse NIH-3T3 cells allowed delineation of several functional subregions within 2.26 kb of the 5'-flanking sequences. The basic promoter element was found to be within the region -128 to -1. In addition, two distinct up-regulatory and two down-regulatory regions were delineated. Deletion analysis of the up-regulatory region between -475 and -129 revealed a strong up-regulatory activity in the fragment extending from -198 to -129, which contains four putative AP2 binding sites. Also, deletion of three of the most 3' SP-1 binding sites dramatically decreased the promoter activity. These findings attest to the complexity of transcriptional regulation of the elastin gene expression in mammalian cells.

LANGERHANS' CELLS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Several reports implicate Langerhans' cells (LC) as susceptible targets, reservoirs, and vectors for transmission of the human immunodeficiency virus (HIV). Other investigators, however, find no evidence for virus infection in the epidermis of HIV-infected patients by immunohistochemical or ultrastructural criteria. To address this controversy, we examined skin from HIV-seropositive subjects at various clinical stages obtained by full thickness biopsy or suction blister. Samples were analyzed for the presence of viral infection by transmission electron microscopy, *in situ* hybridization for HIV-specific mRNA, polymerase chain reaction for HIV-specific DNA, immunocytochemistry, and direct virus isolation by coculture onto macrophage target cells. By these techniques, demonstration of HIV in the epidermis of infected patients was infrequent. In contrast, viral DNA was readily detected from the dermis of the same skin samples. The number and morphology of LC in skin of infected patients were within normal limits by staining for DR and T6 in all stages of disease, except from autopsy specimens. Ultrastructural changes, including vacuolization, were noted in 5/17 samples, but no viral particles were seen in the skin. The role of LC as antigen-presenting immune cells in the pathogenesis of HIV disease is as yet unclear; however these studies suggest that its role as a viral reservoir or vector of transmission is unlikely.

CONNECTIVE TISSUE MAST CELL DEGRANULATION HETEROGENEITY DEFINED BY *IN VIVO* SECRETAGOGUE RESPONSES.

M.S. Kaminer, G.F. Murphy, B. Zweiman, R.M. Lavker, Departments of Dermatology, and Allergy and Immunology, Univ. of Pennsylvania, Philadelphia, Pennsylvania.

Mast cell degranulation is characterized ultrastructurally by two distinctive types of granule alteration. The first involves the entire matrix of each granule (global) and has been most extensively characterized in suspensions of isolated pulmonary mast cells exposed *in vitro* to anti-IgE (so-called "anaphylactic degranulation; AD"). The second involves only zones of individual granules (segmental) and is typical of *in vivo* mast cell degranulation associated with an array of inflammatory disorders (so-called "piecemeal degranulation; PD"). In the present study, early phases of cutaneous mast cell degranulation *in vivo* were examined sequentially after intradermal injection of ragweed extract in i) sensitized atopic individuals (N = 5) and non-atopic controls (N = 4), ii) after cutaneous challenge with dinitrochlorobenzene (DNCB) in pre-sensitized subjects (N = 2), and iii) in one patient clinically anergic for DNCB. Fifteen, 30, 60, and 180 seconds after ragweed challenge, global degranulation of the AD-type was observed in the majority of mast cells of sensitized, atopic subjects. Heterogeneity for degranulation was indicated by juxtaposition of affected granules with approximately equal numbers of unaffected granules in the absence of intermediate forms. Five and 10 minutes after ragweed challenge, the majority of granules were affected by either global changes or by segmental alterations identical to PD. DNCB-induced degranulation sampled at 1, 4, 24, 48, and 96 hours after challenge demonstrated predominantly global granule alterations of the AD-type.

These data suggest that skin mast cells *in situ* i) exhibit global (anaphylactic-type) degranulation during the early phase of type I and type IV hypersensitivity responses, and ii) the ability to detect global (AD-type) or segmental (PD-type) granule changes may relate to chronicity of secretagogue stimulus. "Anaphylactic degranulation" is not solely associated with IgE-mediated events; "piecemeal degranulation" is not exclusive to naturally-occuring, *in vivo* diseases; and granule alterations induced by IgE-dependent stimuli appear to affect granule subpopulations differentially over time. Descriptive terms of global and segmental degranulation appear to more appropriately describe these granule alterations.

MELANOMA DIFFERENTIATION - MODULATION OF THE TUMORIGENIC AND METASTATIC PHENOTYPE.

Koichiro Kameyama, Wilfred D Vieira, Katsuhiko Tsukamoto, Lloyd W Law, and Vincent J Hearing, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland.

Using sublines of the B16F10 and JB/MS murine melanoma cells which are pigmented to varying degrees, we have examined the relationships among differentiation, tumorigenicity, and metastatic potential. All melanoma lines tested grew as metastases, but undifferentiated and amelanotic (JB/MS-w) cells failed to grow as primary subcutaneous tumors; those cells, which had few melanocyte stimulating hormone (MSH) receptors, did not respond to MSH by increasing melanin production, as did the other cell lines. Although *in vitro* treatment with MSH did not affect primary tumor growth in these cell lines, such treatment decreased pulmonary metastases of pigmented (JB/MS and JB/MS-b1) and JB/MS-w cells, but significantly increased pulmonary metastases in poorly pigmented (B16F10 and JB/MS-p) cells. In order to understand the mechanism eliciting these varied MSH effects, the expression of surface melanoma antigens, surface protease activity and susceptibility to NK cells were examined. MSH did not alter surface melanoma antigen expression significantly, but increased the NK cell susceptibility of B16F10, JB/MS and JB/MS-b1 cells. There was an inverse correlation between differentiation and proliferation *in vitro*, and the more pigmented melanoma cells expressed relatively low levels of Class I MHC, relatively high levels of Class II MHC and the highest experimental metastatic capacity. These results demonstrate that MSH possesses the capacity to regulate not only melanogenesis, but also other factors critical to the metastatic growth of melanoma cells.

EXTRACELLULAR LOCALIZATION OF HUMAN CONNECTIVE TISSUE MAST CELL GRANULE CONSTITUENTS *IN VITRO*.

Michael S. Kaminer, Robert M. Lavker, George F. Murphy, Department of Dermatology, University of Pennsylvania, Philadelphia, PA.

Controversy exists as to whether morphologically identifiable externalization of intact mast cell granule contents represents a true form of degranulation. Previous experimental models have relied upon degranulated suspensions of pulmonary mast cells which routinely do not show externalization. In the present study, cultured explants of newborn human foreskin were exposed for 45 minutes to varying concentrations of five different secretagogues - morphine sulfate, calcium ionophore (A23187), compound 48/80, anti-IgE, and substance P. All secretagogues, but not control media and substance P analogue, produced i) degranulation characterized by global loss of granule matrix organization and density, ii) swelling and intergranule fusion to form membrane-bound conduits, and iii) merging of fused granule membranes with mast cell plasma membranes. This last event was associated with extrusion of amorphous granule contents into the pericellular connective tissue. Granule loss was facilitated by apparent incorporation of granule membranes into mast cell plasma membranes to form microvillous processes; empty cytoplasmic vacuoles were infrequently observed. Non-membrane-bound extracellular granule contents could be readily identified by retention of rounded contour and by a characteristic, finely granular substructure that was in focal continuity with actively discharging granules.

The inability of previous *in vitro* studies to document the ultrastructure of mast cell granule externalization during degranulation may relate to subtype of mast cells studied (e.g. pulmonary or mucosal vs. dermal or connective tissue-type) or use of cell suspensions, as opposed to *in situ* models. Skin organ culture offers a useful experimental tool for study of the interactions of mast cell-derived mediators (proteinases, cytokines, proteoglycans) with the extracellular matrix and related cellular components of the cutaneous microenvironment.

DECREASED SUPEROXIDE DISMUTASE ACTIVITY IN THE LESIONAL SKIN OF PSORIASIS.

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We measured superoxide dismutase(SOD) activities in the psoriatic skin by two different methods, electron spin resonance(ESR)-spin trapping method and enzyme immunoassay(EIA). Skin specimens, including epidermis and the superficial dermis, were obtained from the lesional and the adjacent non-lesional skin of psoriatics, and from the discarded skin after skin surgery as controls. Supernatants of the homogenized skin were subjected to the assays. In ESR method, xanthine oxidase was added to the mixture of the specimen, hypoxanthine, and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Thirty seconds later, the ESR spectrum of DMPO-O_2^- spin adduct was analyzed by ESR spectrometer (JES-FE-1X, JEOL, Tokyo, Japan). For EIA, monoclonal anti-human Cu,Zn-SOD antibody (UBE EIA KIT SOD(Cu,Zn), UBE, Tokyo, Japan) was used. The SOD activity in the lesional skin of psoriatics measured by ESR method was 3.81±0.37U/mg protein, which was significantly lower than non-lesional skin of the psoriatics (5.18±0.70U/mg prot.) and the healthy control (6.83± 0.74U/mg prot.). By EIA, Cu,Zn-SOD level in the lesional skin of psoriatics was 1217±230ng/mg prot., and was significantly lower than non-lesional skin of the psoriatics (2688±271ng/mg prot.) and the healthy control (2329±169ng/mg prot.). These results, together with the reports on decreased SOD levels in hyperproliferative conditions, such as tumors, may suggest a possible relationship between the controlling system for active oxygen species and the regulatory mechanism of cell proliferation.

GENETIC BASIS OF LUPUS DERMATOSES IN AUTOIMMUNE-PRONE MRL/lpr MICE.

Hideo Kanauchi, Fukumi Furukawa and Sadao Imamura, Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

MRL/Mp-lpr/lpr (MRL/lpr) mice are well known to involve an autosomal recessive mutant gene "lpr" which produces massive T cell proliferation, lupus nephritis and dermatoses. In human SLE, genetic basis of cutaneous lesions is not clear. To better understand the basis, we designed the genetic studies on lupus dermatoses in MRL/lpr mice.

MRL/lpr, the control MRL/Mp-+/+ (MRL/n), 124 F1 hybrid of (MRL/lpr × MRL/n), 112 F2 hybrid of (F1 × F1) and 78 backcross (BC) of (F1 × MRL/lpr) mice were raised in our laboratory. All mice used in this study were female and 5-mo-old. Lymph-node swelling, proteinuria and skin lesions were carefully checked, and specimens of skin were prepared for immunofluorescent studies (LBT: lupus band test).

Incidences of lupus dermatoses are shown in Table.

	MRL/lpr	MRL/n	F1	F2	BC	
Skin lesion	69.5	0	0	8.1	15.4	(%)
LBT	78.6	0	0	20.5	31.4	(%)

In F2 mice, LBT incidence was almost compatible with theoretical ratio (19.7 %). However, the incidence of skin lesions was much lower than expected ratio (17.4 %). Similar results were observed in BC mice. Chi-square statistical analysis revealed the strong association among LBT, macroscopic skin lesion and lymphoproliferation in F2 and BC mice. Association between lupus dermatoses and proteinuria was weak but significant.

These findings suggest that the appearance of LBT positivity in MRL/lpr mice is regulated by lpr gene (# 5 chromosome), whereas macroscopic skin lesions need other additional modifying factors for the development of lesions.

CHARACTERIZATION OF A MODEL KERATINOCYTE-LYMPHOCYTE ADHESION SYSTEM WHICH IS MODULATED BY GAMMA INTERFERON (IFN-γ). G.D. Karabin, R.S. Mitra, L. Stoolman, B.J. Nickoloff. Depts. of Derm. and Path., Univ. of Mich., Ann Arbor.

The normal inflammatory process in skin is highly regulated with rapid induction followed by termination. The molecular mechanisms responsible for early influx of T cells into the epidermis, and eventual movement out of the epidermis during cutaneous inflammation is unknown. We constructed an in-vitro model system utilizing a T cell line to determine whether keratinocytes (KCs) possess an inherent capacity to initiate and terminate T-cell epidermal cell interactions via intercellular adhesion molecule-1 (ICAM-1) and IL-1 (a T-cell chemoattractant).

A Jurkat cell (JC) derivative (JS9), shown to mimic the endothelial binding characteristics of circulating mature T-cells, was ^{51}Cr labeled, activated with phorbol ester (TPA; $.01\text{-}10^3$nM, 45 mins), then incubated with IFN-γ treated, cultured human KCs grown on collagen-coated coverslips. JC-KC adhesion, KC ICAM-1 and IL-1β mRNA expression were monitored over a range of IFN-γ concentrations ($0.1\text{-}10^3$ U/ml) and incubation times (0.5-96 hrs).

The adhesion of JC to KCs did not significantly occur if the JC were not TPA treated or if KCs were not IFN-γ treated. Adherence was abolished by incubating JC with anti-LFA-1 (TS 1.18) antibody. Optimal JC binding occured after KCs were exposed to 100 U/ml of IFN-γ for 48 hrs. and after JC were treated with 25 nM TPA. During the first 48 hrs, KCs revealed dose dependent parallel increases in both IL-1, ICAM-1 mRNA and functional binding by JCs. However, the ICAM-1 mRNA levels declined in KCs after 48 hrs with a corresponding decrease in JC binding.

These results indicate that KCs can modulate ICAM-1/IL-1 expression and thus, T lymphocyte adhesion in response to IFN-γ. Our model system is consistent with KC-T cell interaction during early inflammation which includes activation of KC-T cell binding accompanied by induction of ICAM-1/IL-1 expression. Later (>48 hrs), KCs repress further induction of ICAM-1 and are refractory to additional T cell adhesion. This system preserves the intrinsic regulatory capacity of KCs and highlights a mechanism for induction/termination of KC-T cell interaction. Finally, this system will potentially permit detection of either T cell/KC gene activation following adhesion.

EVIDENCE FOR OLEIC ACID-INDUCED PHASE SEPARATION WITHIN THE STRATUM CORNEUM. A.Kennedy, M.Francoeur, A.Jakowski, R.Potts, Pfizer,Groton,CT, B.Ongpipattanakul, R.Burnette, University of Wisconsin, Madison,WI.

Fourier transform infrared spectroscopy and electron microscopy were utilized to investigate oleic acid-induced lipid phase separation within the stratum corneum (SC). The results indicate that oleic acid exerts a significant effect on the SC lipids by lowering the transition temperature, and increasing their disorder once they are melted. At 32°C, though, oleic acid did not appreciably alter the disorder of the SC lipids. Oleic acid, itself, at concentrations known to enhance percutaneous absorption was found to be highly disordered from 0 to 100°C. Similar results were obtained for lipids isolated from SC by $CHCl_3$:MEOH extraction. Electron microscopy revealed no gross changes in the bilayer and pointed to the presence of oleic acid between the corneocytes. The coexistence of "melted" oleic acid and "solid" endogenous lipids at physiological temperatures suggest that frank phase separation exists within the SC bilayer. Furthermore, these findings are consistent with the hypothesis that enhanced diffusion of polar permeants may occur through "defects" associated with the interfacial regions of SC phase-separated lipids.

HUMAN WOUND FLUID FROM ACUTE WOUND STIMULATES CELLULAR PROLIFERATION. Matthew H. Katz, Alfred F. Alvarez, William H. Eaglstein and Vincent Falanga. Department of Dermatology & Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida.

The beneficial effect of occlusive wound dressings on the healing process of acute wounds has previously been demonstrated. However, the mechanism is unknown. We examined the effect of wound fluid on cell proliferation of human dermal fibroblasts and umbilical vein endothelial cells. The acute wound fluid (AWF) was sterily collected from underneath a vapor permeable membrane 24 hours after the dressing was placed over donor sites of patients undergoing split thickness skin grafting. Cells were seeded overnight at a density of $2500/cm^2$ in optimal growth media (control). Control media, with or without 2% AWF, was added the next day (day 0) and on day 3. Cells were counted with a hemacytometer on days 0, 1, 3, 4, 6 and 7. The table shows mean cell counts $x10^{-3}$ ± SD of representative days.

CELL TYPE	FIBROBLASTS		ENDOTHELIAL CELLS	
	Control	2% AWF	Control	2% AWF
Day 3	7.7 ± 1.6	13.8 ± 3.6	8.0 ± 3.8	16.3 ± 6.4
Day 6	22.2 ± 4.1	63.7 ± 13.0	51.0 ± 4.9	90.0 ± 14.0

Separation of wound fluid by dialysis showed that the stimulatory effect of unseparated wound fluid persists in fractions greater than 10K MW. These results show that 2% AWF is stimulatory on cell growth of human fibroblasts and endothelial cells. The beneficial effect of occlusive dressings on acute wound healing may be due to the maintenance of contact between the wound and certain mitogenic factors in the wound fluid.

EFFECT OF CYCLOSPORIN A ON THE REGULATION OF SIGNAL TRANSDUCTION MECHANISMS IN CULTURED KERATINOCYTES. L Khandke, R Ashinoff, JG Krueger, J Krane, L Staiano-Coico, R Grossman, D Murphy, R Delaney, AB Gottlieb. Rockefeller Univ. and Cornell Univ. Med. Coll., NY, NY.

Cyclosporin A (CSA), an immunosuppressive cyclic endacapeptide, is effective in the treatment of recalcitrant psoriasis. Previous work suggests that keratinocyte hyperproliferation and inflammation are linked in psoriasis and that immune mechanisms participate in the pathogenesis of psoriasis. We have identified two important growth factors, transforming growth factor (TGF)-alpha and interleukin-6 which are overproduced in psoriasis and which may trigger cellular growth activation. TGF alpha expression was studied in skin sections from psoriasis patients treated with CSA and in neonatal keratinocytes cocultured with CSA and gamma interferon.

Decreased TGF-alpha levels were detected in psoriatic plaques after CSA treatment in vivo, using immunoperoxidase staining with a monoclonal anti TGF-alpha antibody. Neonatal keratinocytes cultured with CSA showed a decrease in cell associated TGF-alpha levels. Northern blot analysis and immunoperoxidase studies demonstrated that gamma interferon increased TGF-alpha expression and that CSA reversed gamma interferon induced increases in TGF-alpha expression. CSA added to growing cultured keratinocytes caused an accumulation of cells within the G_1 phase of the cell cycle. CSA did not promote terminal keratinocyte differentiation as assessed by transglutaminase activity. CSA treatment of normal cultured keratinocytes did not alter EGF receptor numbers or binding affinity. Our results suggest that CSA action on keratinocytes in psoriasis may be mediated by a decreased expression of TGF-alpha.

QUANTITATIVE ANALYSIS OF CERAMIDES IN THE STRATUM CORNEUM OF AGED SKIN AND ATOPIC DERMATITIS. Makoto Kawashima, Kumi Morita, Yuko Higaki, Akira Hidano, Satoru Abe and Genji Imokawa, Department of Dermatology, Tokyo Women's Medical College, Tokyo and Kao Corporation, Biological Science Laboratories, Tochigi, Japan.

Sphingolipids are an important determinant for both water-retention function and permeability barrier in the stratum corneum. However, detailed analysis of ceramides has not been done in skin diseases which show defective water-retention and barrier function. In attempt to assess the quantity of ceramides per unit mass of the stratum corneum in aged skin and atopic dermatitis, stratum corneum lipids were collected by hexane/ethanol extraction of the stratum corneum sheet scraved by cyanoacrylate resin. After separation of cyanoacrylate resin with dimethylformamide, the weight of the stratum corneum mass was measured. The ceramides were quantified by thin layer chromatography and evaluated as ug/mg stratum corneum. In the forearm skin of healthy individuals(n=40), the total ceramide content significantly declined with increasing age. Each of six ceramide fractions simultaneously decreased with aging. In atopic dermatitis (n=30), there was a marked reduction in the amount of ceramides in involved forearm skin as compared with those of healthy individuals of the same age. Interestingly, uninvolved skin also exhibited a similar and significant decrease of ceramides. Among six ceramide fractions, ceramide I was mostly reduced in both involved and uninvolved skin. These findings suggest that an insufficiency of ceramides in the stratum corneum is an etiologic factor in atopic dry skin.

DIFFERENTIAL EXPRESSION OF TRANSGLUTAMINASE IN NORMAL HUMAN EPIDERMIS. Hee Chul Kim[1], Maria Turner[2], William Idler[2], Sang Chul Park[3], Soo I Chung[1], and Peter Steinert[2]. Laboratory of Cellular Development and Oncology, NIDR[1] and Dermatology Branch, NCI[2], NIH, Bethesda, MD, and College of Medicine, Seoul National University, Seoul, Korea[3].

Understanding the expression of different transglutaminases (TGase C, E and K) and their substrate specificities toward cellular proteins in various stages of keratinocyte differentiation in epidermis may provide insight into biochemical mechanism of cell envelope formation. Northern blot analysis of human foreskin with a cDNA probe encoding TGase K showed three bands equivalent to 2.9, 3.3 and 3.7 kb, indicating the presence of three different messenger RNAs corresponding to TGase K, E and C respectively. However, examination of TGases by the enzymatic and immunological methods showed that TGase E (the only TGase expressed as a zymogen) was the predominant one in the soluble extract of human foreskin but was not detected in primary cultures of keratinocytes. TGase K was present in all of the non-extractable particulate fractions. Two glutamine-rich N-terminal (2-19) and C-terminal (298-316) peptides of Loricrin (the human glycine-rich cornified envelope precursor protein) were found to be far better substrates for activated TGase E than for membrane associated-TGase K. Immunohistological staining of human epidermis with anti-TGase E and anti-N-terminal peptide of Loricrin showed that both antigens are localized mainly in the stratum granulosum and stratum corneum of normal human epidermis. These data suggest that TGase E, by its preferential reactivity and coincidental its expression with Loricrin, may be responsible for the cross-linking of Loricrin during cell envelope formation.

IN VITRO 5-BROMO-2-DEOXYURIDINE UPTAKE OF CULTURED ATOPIC MONOCYTES. Jin-Wou Kim, Seth R. Stevens, Sai C. Chan, Jon M. Hanifin. Dept. of Dermatology, Oregon Health Sci. U., Portland, Oregon

Previous studies have shown altered cyclic nucleotide metabolism in monocytes (MO) from patients with atopic dermatitis (AD). We questioned whether this would effect basic cellular physiology. Therefore we evaluated proliferative and metabolic activity in MO from patients with AD compared to normal (NL) subjects. By using cytokines, we are able to maintain low-grade proliferation of blood-derived monocytes. In this study, we have quantitated relative DNA and protein synthesis as well as oxygen consumption.

Histopaque-isolated venous blood mononuclear cells were cultured in 5% AB serum in RPMI 1640 in tissue culture chamber/slides. AD MO were pulsed with 20 μM BrdU for 48 hours to measure the proliferative activty. On days 3 and 7 adherent and suspension MO were harvested and immunocytochemically stained with anti-BrdU and avidin-biotin peroxidase labeling. U937 human histiocytic lymphoma cell line was used as the positive control. Leukocyte oxygen consumption was measured with an oximeter and protein synthesis by spectrophotometry.

BrdU uptake was increased at day 7 as compared to day 3 in both AD and NL MO, but there was no significant difference on either day between AD and NL MO cultures. At day 3, mean percentages of BrdU uptake of 4 pairs of AD and NL adherent MO were 0.33±0.19% and 0.23±0.12% respectively. At day 7, BrdU uptake of 4 pairs of AD and NL adherent MO were 3.5±0.8% and 4.6±2.4% respectively. Suspension cells were roughly equivalent in uptake compared to adherent cells. The oxygen consumption curves and protein synthesis data showed no difference between AD and NL leukocytes.

These studies show the feasibility of using BrdU to measure PA in cultured MO. We have been unable to detect differences in unstimulated PA of MO cultures from patients with AD and NL. The normal PA of non-stimulated AD cultured MO might reflect an intracellular homeostatic mechanism in AD.

CLUSTER FORMATION BY ANTIGEN-BEARING DENDRITIC CELLS IN THE LYMPH NODES OF CONTACT SENSITIZED MICE Ian Kimber, Marie Cumberbatch and Ian Illingworth, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ UK.

Topical sensitization of mice results in a rapid accumulation of dendritic cells (DC) in lymph nodes draining the site of exposure. These DC express MHC class II determinants and a significant proportion bear high levels of antigen. Highly-enriched populations of DC prepared from the lymph nodes of contact sensitized mice stimulate specific T lymphocyte proliferation in vitro and effectively transfer sensitization to naive recipients.

During the initiation of immune responses DC form clusters with T lymphocytes. We have developed methods to examine whether antigen-bearing DC associate with T cells in vivo and in vitro. A significantly higher percentage (70%) of antigen-bearing DC were found to have formed clusters with T lymphocytes in vivo compared with DC with no detectable antigen (30%). No clusters between DC and T lymphocytes were found in the lymph nodes of naive mice. In vitro experiments revealed that antigen-bearing DC formed clusters with lymphocytes from both naive and skin-sensitized mice. DC from naive mice failed to associate with T cells in vitro.

These data are compatible with DC playing an important role not only in the transport of antigen from the skin, but also the presentation of that antigen to T lymphocytes.

CHARACTERIZATION OF AN INHIBITOR OF IL-1 DEPENDENT T CELL ACTIVATION PRESENT IN PSORIATIC, BUT NOT NORMAL, SKIN. Nack In Kim, Craig Hammerberg, Gary Fisher, John J. Voorhees, and Kevin D. Cooper, Dept. of Dermatology, Univ. of Michigan Medical School, Ann Arbor, MI.

We have recently demonstrated that reduced Interleukin-1 (IL-1) activity in psoriatic lesions was in part due to the presence of an IL-1 inhibitor. This inhibitory material has now been purified by Fast Protein Liquid Chromatography (FPLC) and its functional activity characterized. The epidermal IL-1 inhibitor isolated from the cytosol of untreated psoriatic epidermis consistently demonstrates a molecular weight of 30 kD (n=4). It has not been observed in normal epidermis (pool of 3 individuals). Despite their similarity in molecular weight, the inhibitor is not TGFβ, since experiments with a neutralizing monoclonal antibody against TGFβ failed to remove the activity. The inhibitor is capable of partially inhibiting IL-1 activity four hours after exposure of the IL-1 responsive cell line, LBRM.33, to a pulse and wash-out of IL-1 and PHA. This suggested an action on post receptor-binding events, since the activity of the inhibitor is not dependent on blocking the IL-1 receptor or binding to IL-1 itself. The inhibitor is not generally suppressive of proliferation; it does not inhibit the response of an IL-2 dependent cell line, HT-2, to IL-2, or the proliferation of dermal fibroblasts or a squamous carcinoma cell line. In addition, stimulation of human mononuclear cells with a specific antigen (tetanus toxoid) is not affected by the inhibitor, whereas proliferation in response to triggering of the T cell activating molecule, CDw60, was completely inhibited, demonstrating selectivity in its effects on T cell activation. Based upon the molecular weight and antibody neutralization experiments, the IL-1 inhibitor isolated from the cytosol of psoriatic epidermis is not like other known IL-1 inhibitors present in the epidermis or elsewhere. The inhibitory activity is restricted since it does not affect cell proliferation in general or all forms of T cell activation. The presence of the inhibitor in psoriatic, but not normal, epidermis suggests that the presence of the inhibitor may result in a selective propagation of T cell subsets activated by inhibitor-resistant mechanisms.

CORTICOSTEROIDS BLOCK KERATINOCYTE IL 6 SYNTHESIS. Reinhard Kirnbauer*, Andreas Köck*, Elisabeth Förster*, Thomas Schwarz*, Thomas A. Luger*,**, *Dept.Derm. II, Univ.Vienna and LBI-DVS, Lab. Cellbiol., Vienna; *Dept.Derm., Hosp.Lainz, Vienna, Austria; **Dept.Derm., Univ.Münster, FRG

The antiinflammatory effect of drugs such as corticosteroids is known to include downregulation of the production of immunomodulating cytokines. Since epidermal cells are able to produce different cytokines including the multitargeted mediator interleukin 6 (IL 6) the present study was performed to investigate the effect of hydrocortisone, dexamethasone and prednisolone on keratinocyte IL 6 production. The human epidermoid carcinoma cell line KB was treated with UVB (100 J/m^2), IL 1 (1 U/ml) or left untreated and incubated for 24 hr with different doses of corticosteroids (10^{-5} to 10^{-12} M.) Supernatants were tested for IL 6 activity using the IL 6 dependent hybridoma cell line B9. All corticosteroid preparations tested blocked constitutive as well as UVB and IL 1 induced KB cell IL 6 production in a dose dependent manner. In contrast, the non-active steroid prednisone did not affect IL 6 production. Northern blot analysis using an IL 6 cDNA probe showed a dose dependent decrease in the steady state level of IL 6 mRNA when cells were stimulated with UVB or IL 1 in the presence of various steroid concentrations (10^{-7}-10^{-9} M). These data indicate that corticosteroids may block KB cell IL 6 production at the level of transcription. Therefore the antiinflammatory effect of corticosteroids in various skin diseases may be partly due to the downregulation of IL 6 in keratinocytes.

HERPES GESTATIONIS: IMMUNOPRECIPITATION AND INDIRECT IMMUNO-ELECTRON MICROSCOPY SHOW SPECIFIC IMMUNOLOGIC SIMILARITIES TO BULLOUS PEMPHIGOID. Soo-Chan Kim, Diya Mutasim, Lynne H. Morrison, Ramsy S. Labib, and Grant J. Anhalt. Departments. of Dermatology, the Johns Hopkins Univ., Baltimore, MD and the Oregon Health Sciences Univ., Portland, OR.

Immunoprecipitation (IP) studies have shown that almost all Bullous pemphigoid (BP) sera recognize a 230 KD antigen (Ag) but most also faintly precipitate a lower MW 166 - 180 KD Ag. In Herpes gestationis (HG), a single prior immonoblotting study (Morrison et al, J Clin Invest. 81:2023-2026, 1989) showed primary recognition of a similar 166 - 180 KD Ag (8/9) and a less frequent detection of the 230 KD Ag (1/9). A large sampling of HG sera have not previously been compared to BP sera by identical techniques.

We obtained 27 sera from patients with HG, 8 control sera from normal term pregnancies and an index BP serum known to IP both the 230 and 166 - 180 KD Ags. All were tested for HG factor by indirect complement fixation (ICF), and Ag detection by immunoprecipitation (IP) using ^{14}C - labelled human keratinocytes extracted in 0.5% NP-40 / 2 mM PMSF / 0.01 M Tris HCl, pH 7.2 according to Stanley et al (Cell.24:897-903,1981).

24 / 27 HG sera were positive for the HG factor by ICF, and of the positive sera, 3 / 24 precipitated both the 230 KD and the 180 KD Ags, 18 / 24 precipitated only the 180 KD Ag, and 3 did not IP any specific Ag. Of the three HG sera that were negative for HG factor by ICF, two precipitated either the 230 KD or the 180 KD Ag; the third was non-reactive by IP. The 230 and the 180 KD Ags precipitated by HG sera comigrated precisely with the corresponding Ags immunoprecipitated by the index BP serum. All control sera were non-reactive by both ICF and IP.

A single serum had high titer IgG, sufficient to perform indirect immuno-EM. This showed binding of HG IgG overlying the cytoplasmic plaque of the basal cell hemidesmosome and the underlying lamina lucida - a pattern identical to that observed with BP sera.

This study demonstrates the close immunologic similarity of BP and HG by specific and sensitive criteria, but unlike BP, there is preferential immunoprecipitation of the lower MW 166 - 180 KD Ag by autoantibodies in Herpes gestationis.

TRANSLOCATION OF LIPOCORTIN I FROM CYTOPLASM TO PLASMA MEMBRANE IN PSORIATIC KERATINOCYTES AND IN LOW TO NORMAL Ca^{2+}-GROWN KERATINOCYTES Yasuo Kitajima, M.Koji Owada* and Hideo Yaoita. Dept.of Dermatology Jichi Med. School, Tochigi, Inst.of Molecular and Cellular Biol. for Pharmaceutical Science, Kyoto Pharmaceutical Univ. Kyoto, Japan

Lipocortin I(LPC-I) is a 35 KD protein which binds phospholipids (PL), and actin in a Ca^{2+}-dependent manner, a major substrate of EGF receptor/tyrosine kinase and C-kinase, and a putative inhibitor of phospholipase(PLpase) A_2. It is distributed in cytoplasm and also bound to plasma membrane. Keratinocytes(KC) in low Ca^{2+}(<0.1mM) medium lack desmosomes, and proliferate without differentiation, but form desmosomes and differentiate in normal Ca^{2+}(>0.1mM) medium. Raising Ca^{2+} level in the medium has been shown to cause a rapid breakdown of inositol PL, and C-kinase activation in KC. Besides, psoriatic epidermis shows an elevated activity of PLpase C and PLpase A_2.

We studied the distribution of LPC-I in cultured human KC and psoriatic epidermis by immunofluorescence microscopy using antibodies to whole molecule, N-terminal fragment and Ca^{2+}-bound form of LPC-I. At normal Ca^{2+} level, LPC-I was detected at cell-cell contacts of the plasma membrane of KC, while it was in the cytoplasm at low Ca^{2+} level. By 30 min after Ca^{2+}(1.87mM) or TPA(10ng/ml) addition, LPC-I seemed to translocate from cytoplasm to plasma membrane. In biopsy, psoriatic KC revealed only membrane-bound LPC-I which was Ca^{2+}-bound form, while normal and nonlesional KC showed a cytoplasmic distribution in suprabasal cells. These results suggest that translocation of LPC-I from cytoplasm to plasma membrane may be involved in KC differentiation, and abnormal keratinization in psoriasis.

THERAPEUTIC IMPLANTS FOR DELIVERY OF CHEMOTHERAPEUTIC AGENTS IN THE TREATMENT OF VETERINARY NEOPLASMS Barbara Kitchell, Charles Liskey, Laure Woods, Edward Luck, Dennis M. Brown Special Veterinary Services, Berkeley, CA, Humphrey Giacopuzzi Veterinary Group, Somis, CA and Matrix Pharmaceutical, Inc., Menlo Park, CA

The safety and efficacy of intralesional delivery of therapeutic agents in Therapeutic Implants (TI) was evaluated in spontaneously occurring tumors in 216 animals. TI consist of a carrier matrix, a vasoactive modifier and a therapeutic agent (e.g. 5-fluorouracil, 5-FU or cis-platinum, cis-DDP). TI were administered to 638 tumors representing 23 discrete diseases; the most predominant neoplasm was squamous cell carcinoma (SCC).

Animals with recurrent disease and/or poor prognosis with conventional therapies were selected. Histologically confirmed tumors were treated with TI containing 5-FU (30 mg 5-FU/ml TI) at weekly intervals for up to 8 weeks or until complete response occurred. If no objective response (≥50% volume reduction) was noted after three 5-FU TI then cis-DDP TI (1 mg cis-DDP/ml TI) were used for up to 8 weeks.

An average of three TI over a 10 week period produced a complete tumor response; 72% of tumors had ≥50% tumor volume reduction and 45% a complete resolution. Patients (108) with resolved tumors had an average disease free interval of 46 weeks to date. Acute cutaneous reactions were limited to 1.6% (33/2021) of TI.

Treatment of spontaneous cutaneous tumors with TI was technically feasible, had minimal side effects and was well tolerated by all patients. TI present opportunity for more effective local lesion therapy without treatment limiting tissue toxicity, for treatment of lesions in anatomical locations inaccessible to invasive procedures, or when tissue conservation is essential.

QUANTITATIVE ASSESSMENT OF ELASTIN AND FIBRONECTIN IN TRETINOIN TREATED PHOTOAGED HAIRLESS MOUSE SKIN. Lorraine Kligman, Frederick Cruickshank, James Mezick and Elaine Schwartz, Depts. of Dermatology, University of Pennsylvania, Philadelphia, PA, and Mt. Sinai School of Medicine, New York, NY and R.W. Johnson Pharm. Research Institute, Raritan, N.J.

Previous studies have demonstrated that topical tretinoin treatment of photoaged mice accelerated the formation of a subepidermal zone of new connective tissue consisting mainly of collagen. The aims of this study were to localize and quantify elastin and fibronectin in the skin of tretinoin treated hairless mice. Animals were irradiated thrice weekly for 10 weeks with 4 minimal erythema doses of UVB from Westinghouse FS 40 bulbs. They were then treated with either 0.05% tretinoin, the ethanol-propylene glycol vehicle, or nothing for up to 10 weeks. Antibodies against soluble elastin and fibronectin were used in immunofluorescence microscopy and immunobinding assay procedures. Elastin and fibronectin were visualized in the subepidermal zone as fine fibrils in the 10 weeks tretinoin treated skins. Extracts prepared from treated and untreated skins were applied to nitrocellulose and incubated sequentially with specific antibodies and ^{125}I-Protein A. Quantification was obtained by densitometric scanning of the autoradiogram. The tretinoin treated skins had a three-fold higher content of soluble elastin than vehicle or untreated mice. In contrast, the content of insoluble elastin (determined by desmosine analysis) was similar in all treatment groups. These results suggest that tretinoin can stimulate new elastin synthesis.

CHOLESTEROL SULFATE AS A MEMBRANE BILAYER STABILIZER. Neil Kitson, Kim Wong, and Pieter Cullis, Div. of Derm., Dept of Med. and Dept of Biochem., U.B.C., Vanc, B.C.

We examined the hypothesis that the amphipathic properties of cholesterol-3-sulfate might promote membrane stability. Using hydrated model membranes composed of egg sphingomyelin (ESPM), a "non-bilayer" phosphatidylethanolamine (DOPE), cholesterol (CH) and cholesterol sulfate (CS), it was found that inclusion of both cholesterol and cholesterol sulfate in equimolar mixtures with ESPM resulted in ^{31}P-NMR lineshapes consistent with liquid crystalline bilayers. DOPE:ESPM 6:1 (mole ratio) underwent a lamellar-to-inverted hexagonal transition at 35°C. Addition of CH (DOPE:ESPM:CH 6:1:1) lowered this transition temperature, whereas addition of an equivalent mole fraction of CS raised it. The effect was insensitive to pH over the range 4.0-8.0, but was sensitive to the addition of calcium ions. We therefore confirm that at least in model membranes capable of transitions from the bilayer to the H_{II} phase under the influence of temperature, CS can act as a membrane stabilizer. In contrast, CH promoted "non-bilayer" phases under the same conditions. We speculate that the high CS/CH mole ratio found in epidermal lipid of patients with recessive X-linked ichthyosis may stabilize bilayer structures in the stratum corneum intercellular space.

MSHα AND ACTH PRODUCTION BY HUMAN KERATINOCYTES: A LINK BETWEEN THE NEURONAL AND THE IMMUNE SYSTEM. Andreas Köck, Elisabeth Schauer, Thomas Schwarz, Thomas A.Luger, LBI-DVS, Lab.Cellbiol. and Dept. Derm.II, Univ.Vienna; Dept.Derm., Hosp.Lainz, Vienna, Austria; Dept.Derm., Univ.Münster, FRG.

There is strong evidence for a close relationship between pituitary derived neuropeptides such as ACTH and melanocyte stimulating hormone alpha (MSHα) and the immune system. In addition, the epidermis is well known to be an active element of the immune system because of the presence of immunocompetent cells and the immunosecretory capacity of keratinocytes. Therefore it was investigated whether keratinocytes can serve as a source of ACTH and MSHα. Supernatants derived from PMA stimulated normal human keratinocytes (HNK) and carcinoma cell lines (A431, KB) which were tested in RIAs specific for MSHα or ACTH contained significant amounts of MSHα and ACTH. Immunoprecipitation of in vivo labeled keratinocytes using monoclonal antibodies against ACTH and MSHα resulted in distinct bands at 2, 4, 36 and 42 kD. Northern blot analysis using a cDNA probe for the MSHα/ACTH precursor proopiomelanocortin (POMC) revealed the expression of POMC specific mRNA in HNK, KB and A431 cells. The production of MSHα and ACTH could be significantly upregulated both at the protein and mRNA level after stimulation with PMA, UV light, IL 1 or IL 4 but not with LPS, GM-CSF, IL 6 or IFNγ. These data provide first evidence that human keratinocytes produce the neuropeptides MSHα and ACTH and thereby may influence the skin immune system as well as melanocyte functions.

TGF-α AUTOINDUCTION REQUIRES EGF RECEPTOR ACTIVATION. SB Klein, J Mendelsohn[1], JJ Vorhees, JT Elder, Dept. Dermatology, Univ. of Michigan, Ann Arbor, MI, and [1]Sloan Kettering Cancer Res. Ctr., New York, NY.

Transforming growth factor-α (TGF-α) is overexpressed in psoriasis and other epithelial neoplasias, and appears to be the endogenous epidermal ligand for the epidermal growth factor receptor (EGFR). TGF-α is autoinduced in keratinocytes (KC), and is a potent stimulus for KC growth. Many of the pleiotypic effects of EGF are mediated by the tyrosine kinase activity of the EGFR, which is negatively regulated by protein kinase C (PKC). Since PKC activity is reduced in psoriatic lesions, deficient negative regulation of the EGFR tyrosine kinase may cause increased TGF-α expression and epidermal hyperplasia in psoriasis. Therefore, it is important to demonstrate that TGF-α autoinduction depends upon EGFR activation.

Normal adult human KC (NHK) were grown in modified MCDB 153 medium (KGM, Clonetics) and rendered quiescent at 50% confluence by 48h incubation in basal medium. Cells were then treated with a variety of growth factors and cytokines, including TGF-α, insulin, IGF-1, BPE, IL-1α, IL-6, bFGF, TGF-ß, GM-CSF, and PDGF. After 4 hours, total RNA was isolated and analyzed for TGF-α transcripts by RNA blot hybridization and densitometry. Of the cytokines tested, only TGF-α significantly stimulated TGF-α mRNA levels (3.58±0.78-fold relative to basal medium control, n=3; p<.0008 vs. other cytokines tested.) The specificity of the autoinductive response was strongly suggestive of EGFR activation. In order to confirm that hypothesis, quiescent KC were treated for 4 hours with 20 ng/ml TGF-α, 20 ng/ml EGF, or 20 nM tetradecanoyl phorbol acetate (TPA) in the presence or absence of the EGF receptor blocking antibody 225 IgG (20-50 nM). Pretreatment (30-60 min) with 225 IgG resulted in marked reduction in the mRNA response to either EGF (2.8-fold vs. 6-fold) or TGF-α (2-fold vs. 5.2-fold), but did not detectably reduce the induction of TGF-α by TPA (4.9-fold vs 4.8-fold). Taken together, these two results indicate that TGF-α autoinduction occurs through the EGF receptor pathway in NHK, and support the hypothesis that inappropriate regulation of EGFR tyrosine kinase activity is central to the pathogenesis of psoriasis.

SYSTEMATIC UNDERREPORTING OF CUTANEOUS MALIGNANT MELANOMA IN MASSACHUSETTS: IMPLICATIONS FOR NATIONAL INCIDENCE FIGURES H Koh R Clapp, J Barnett, M Prout, A Geller, R Lew Departments of Dermatology and Medicine and Section of Epidemiology & Biostatistics Boston University Schools of Medicine and Public Health Boston, MA

The national cancer reporting system, the Surveillance, Epidemiology and End Results (SEER), relies only on hospital-supplied data to generate cancer incidence figures. Because cutaneous melanoma is increasingly diagnosed and treated in outpatient settings, we hypothesized that melanoma incidence rates are underreported. We used an independent tracking system in Massachusetts by contacting melanoma pathologists about newly-diagnosed cases and then matching these cases against those in the official state cancer registry from 1982-1986. Of the 1345 compiled melanomas, 457 matched with Massachusetts Cancer Registry records. Of the remaining 888, 446 did not meet stated inclusion criteria and were eliminated. Of the remaining 442, we reconfirmed 364 as Massachusetts incident cases not in official registry records. After adding those 364 to the 2666 cases already in the registry, we found that at least 12% (364/3030) and perhaps as many as 20% of new cases of cutaneous malignant melanoma in Massachusetts are not recorded in the cancer registry, significantly higher than the expected 5% (p=0.0001). This trend will worsen over time. We conclude that the true incidence of cutaneous malignant melanoma in Massachusett and probably the United States is significantly higher than reported.

THE REACTION OF SKIN TYPE V TO ULTRAVIOLET RADIATION. N. Kollias, A.H. Baqer, Y.A. Malallah, H. Al-Ajmi, and I. Sadiq, Department of Dermatology, Massachusetts General Hospital, Boston, MA and Al-Sabah Hospital, Ministry of Public Health, Kuwait.

The purpose of this investigation was to study the inflammatory and pigmentary reactions of skin type V to ultraviolet radiation. The minimum erythema dose (MED) of 20 healthy volunteers was determined at 295, 305 and 315 ±5 nm in increments of $\sqrt{2}$; reaction was assessed 24 hours post irradiation. Also 14 volunteers were tested with 305 ±5 nm and 365 ±25 nm. The 365 nm MED was determined using a series of doses 150-350 J/cm^2 in increments of 50 J/cm^2. The 365 MED was determined using a diffuse reflectance spectrophotometer because of the very large immediate pigment darkening (IPD) reaction. The minimum melanogenic dose (MMD) was determined visually 7 days post irradiation. In the first study it was determined that the 295 nm MED was 47 mJ/cm^2, the 305 MED was 150 mJ/cm^2 and the 315 MED was 1800 mJ/cm^2. A significant finding of this study was that while all volunteers pigmented as a result of the exposures, some generated erythemas at doses not unlike those of skin types II or III. In the second study the 305 nm MED was 175 mJ/cm^2 and the 365 nm MED 257 J/cm^2. The 305 nm MED was found to be 237 mJ/cm^2 and for 365 nm was 90 mJ/cm^2. The minimum exposure to generate an IPD reaction to 365 nm was 14 J/cm^2. It was found that the erythema action spectrum for type V skin is the same in form as for types III and IV but shifted down by a factor of 2.2. While all type V individuals pigment as a result of a UV insult some burn, some burn mildly and some never burn.

ORAL ISOTRETINOIN PREVENTION OF SKIN CANCER IN XERODERMA PIGMENTOSUM: INDIVIDUAL VARIATION IN DOSE RESPONSE Kenneth H. Kraemer, John J. DiGiovanna and Gary L. Peck National Cancer Institute, NIH, Bethesda, MD

We have previously shown that high dose (2.0 mg/kg/day) oral isotretinoin was effective in decreasing the rate of new skin cancer formation in xeroderma pigmentosum (XP) patients but was very toxic. In order to determine if chemoprevention could be attained with less toxicity, 7 XP patients were treated with low dose (0.5 mg/kg/day) isotretinoin. Five of these patients had completed a 2 year high dose treatment course with an average reduction in skin cancers of 63 percent (N Engl J Med 318:1633, 1988). However all patients had mucocutaneous toxicity and 2 had laboratory toxicity which required dose reduction. When the drug was discontinued, the tumor frequency increased a mean of 8.5-fold over the frequency during treatment.

During 8-26 months of low dose treatment, the frequency of skin cancers decreased in 5 of 7 patients in comparison to the interval without treatment. Four of these patients had a dose response where the skin cancer frequency on low dose was intermediate between high dose and no treatment. One patient had a similar good response to both doses. In contrast, 2 patients did not respond to the low dose. In all patients mucocutaneous toxicity and laboratory abnormalities were less severe with the low dose treatment. This study indicates that the lowest effective, least toxic chemopreventative dose of isotretinoin varies among XP patients.

AFFINITY PURIFICATION AND CHARACTERIZATION OF RABBIT ANTIBODIES AGAINST MOLECULARLY-DEFINED EPITOPES ON THE BULLOUS PEMPHIGOID ANTIGEN (BPA). Neil J. Korman, Toshihiro Tanaka, Vera Klaus-Kovtun and John R. Stanley, Dermatology Branch, NCI, NIH, Bethesda, MD.

We have molecularly cloned BP cDNA encoding a peptide of 160 kD ending at the carboxy (C)-terminus of the 230-kD BPA. The goals of this study were to raise, affinity purify, and characterize rabbit antibodies against localized epitopes of BPA defined by this cDNA. We immunized rabbits with 3 β-galactosidase fusion proteins (FP) from near the C-terminal end of the BPA and with synthetic peptides (of 16-19 amino acids) from several areas along the BPA. Rabbits immunized with 2 of 3 FP developed antibodies that bound, by immunofluorescence (IF), the roof of 1M NaCl split skin. One of these IF positive antibodies recognized the native 230 kD BPA by immunoprecipitation (IP); whereas both IF positive antibodies bound the denatured BPA by immunoblotting (IB). Three of 8 synthetic peptide immunogens resulted in high titer BP antibodies that bound the roof of 1M NaCl split skin and recognized the native BPA by IP. Of the 5 antibodies not recognizing native antigen by IF or IP, 2 bound hidden sites exposed on denatured BPA by IB. High titer antibodies raised against one FP and 3 synthetic peptides (all IF and IP positive) were affinity purified by passage over columns containing the bound immunogen. IF studies revealed that affinity purified antibodies were usually detectable to an endpoint of < 10 μg /ml. Immunoelectron microscopy, utilizing affinity purified antibodies against synthetic peptides from near the C-terminal end of the BPA, showed specific binding to the inner plaque of hemidesmosomes at the site of keratin filament insertion. These studies demonstrate that: 1) affinity purified, monospecific antibodies can be produced against molecularly-defined epitopes on BPA; 2) certain epitopes of BPA are immunogenic in rabbits but hidden in the native molecule, and therefore are probably not important in disease pathophysiology; and 3) epitopes on the C-terminal end of BPA localize to the hemidesmosome plaque where keratin filaments insert.

INCREASED EXPRESSION OF PDGF RECEPTORS IN PSORIASIS AND GROWTH-ACTIVATED SKIN WOUNDS. JF Krane, DP Murphy, AB Gottlieb, DM Carter, CE Hart, MJ Murray, and JG Krueger Rockefeller Univ., NY, NY 10021 and Zymogenetics, Seattle, Wa 98105

Mitogenic effects of PDGF are produced by binding of PDGF dimers to PDGF receptor alpha or beta subunits. We have studied the expression of the PDGF receptor in cryostat sections of normal and growth-activated human skin using a monoclonal antibody (PR7212) specific for the beta subunit. Compared to normal skin, chronic skin wounds from 15 patients showed a pattern of growth activation using a panel of markers. PDGF receptors were expressed at low levels in normal skin, with only occasional staining of dermal connective tissue cells. In contrast, PDGF receptors were greatly elevated in fibroblasts and blood vessels in the dermis of chronic wounds. PDGF receptors were also increased in dermal fibroblasts and vascular elements in active psoriatic skin, whereas normal-appearing skin from the same patients had low-level PDGF receptor expression. PDGF receptors were not detected in normal, psoriatic, or growth-activated epidermis. To confirm specificity of PR7212 immunoreactivity, the production of PDGF receptors was assessed by functional and radioimmunoprecipitation assays in normal human keratinocytes and normal human skin fibroblasts in culture. Fibroblasts, but not keratinocytes, expressed abundant PDGF receptors which could be down-regulated by rhPDGF, and immunoprecipitation experiments showed synthesis of a 180 kd beta subunit receptor protein. Differential expression of PDGF receptors could regulate increased proliferation of vascular and connective tissue cells observed in psoriasis and chronic skin wounds. The lack of epidermal PDGF receptors suggests human keratinocyte proliferation is not directly regulated by PDGF.

EXPRESSION OF PROTEIN KINASE C ISOZYME IN EPIDERMAL LANGERHANS CELLS OF THE MOUSE. Yoh-ichi Koyama, *Takahisa Hachiya, *Masatoshi Hagiwara, **Miya Kobayashi, Koji Ohashi, **Takeshi Hoshino, *Hiroyoshi Hidaka, and Tohru Marunouchi, Division of Cell Biology, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake; *Department of Pharmacology, **Department of Anatomy, Nagoya University School of Medicine, Nagoya, Japan.

Protein kinase C (PKC) is encoded by a complex of a gene family, and its multiple isozymes are expressed in various mammalian tissues. We examined whether PKC is expressed in epidermal Langerhans cells (LCs) of the mouse by using monoclonal antibodies specific to PKC I, PKC II, and PKC III isozymes. Immunohistochemical studies revealed that LCs of adult C57BL/6 mice express PKC II, while PKC I and PKC III were not detected. Neither isozymes were detected in keratinocytes. For biochemical analysis of PKC isozymes, enzymatically dissociated epidermal cells were fractionated into LC-enriched (Ia^+>97%) and LC-depleted (Ia^+<0.1%) epidermal cell populations. Immunoblot analysis revealed that LCs express only PKC II among three isozymes. Neither isozymes were detected in the LC-depleted epidermal cell population. These results suggest that PKC II isozyme is a novel marker of LCs and that it possibly plays a regulatory role in epidermal LCs of the mouse in vivo.

FIBROBLAST CONDITIONING OF ACELLULAR RETICULAR HUMAN DERMIS FACILITATES ITS EPITHELIALIZATION. N. Krejci, R. Langdon, C. Cuono*, J. McGuire, Dpts. of Dermatology and *Surgery, Yale University, School of Medicine, New Haven, CT.

This study investigates conditions influencing keratinocyte outgrowth on superficial reticular dermis (SRD) in vitro. This was prepared by taking a second cut (0.5mm depth) after harvesting a standard split thickness skin graft from a human cadaveric skin donor. We studied native living dermis as well as dead acellular dermis (prepared by freeze thawing x3, 5000 cGy gamma irrad. and 3 weeks soaking in PBS at 37°o). The acellular dermis was conditioned by one of two methods: (a) application of the matrix molecules collagen type IV or fibronectin (both 0.2ug/cm^2) or (b) application of cultured human dermal fibroblasts (5x10^{4}0/cm^2) followed by 5 days of culture in DMEM with 10% bovine calf serum. These dermal preparations were tested for their ability to support keratinocyte outgrowth from neonatal foreskin explants and from focally applied cultured human keratinocytes (4x10^4 cells restricted to a 0.3cm^2 area). Explants placed on dead, acellular dermis did not exhibit keratinocyte outgrowth. Further, conditioning this dermis with matrix molecules did not influence outgrowth. In contrast, acellular dermis on which fibroblasts had been cultured, supported outgrowth from explants. The epidermis exhibited stratification, cornification and suprabasalar mitoses. Interestingly, native reticular dermis, despite the presence of resident fibroblasts, did not support epithelialization. Results obtained with cultured keratinocytes paralleled our findings with explants.

EFFECTS OF IL-8 ON EPIDERMAL PROLIFERATION. G Krueger, C Jorgensen, C Miller, J Schroeder, M Stiecherling, E Christophers. Dermatology, Univ of Utah, USA, and Univ of Kiel, West Germany.

Cytokines of physiologic importance that act in a paracrine fashion (message over distance) are those that cause tissue change, e.g., epidermal proliferation. Presence of IL-8 in lesions of psoriasis, its production by monocytes and fibroblasts, and its effects on neutrophils led to experiments to elicit its effects on epidermal proliferation. The assay used both an early analysis of DNA synthesis by keratinocytes and a terminal analysis of the volume of epidermis (thickness x area). Keratinocytes were seeded on a Millicell HA membrane and grown under conditions that permit both proliferation and differentiation. Effects were analyzed by adding IL-8, EGF, and IL-1, either singly or in combination with control and monoclonal antibodies, to IL-8. Results show: 1) IL-8 and IL-1 enhance DNA synthesis; anti-IL-8 impairs this response. 2) The volume of epidermis generated by day 17 is altered by these combinations. Specifically, IL-8 increased epidermal volume by 1.82 X and IL-1 by 1.1 X. Singly, EGF caused no increase; however, maximal volume was noted with the combination of IL-8 ($1x10^{-10}$ g/ml) and EGF ($1x10^{-8}$ g/ml); 2.45 X volume without either IL-8 or EGF. Anti-IL-8 inhibited the IL-8 effect, while the control monoclonal antibody had no effect.

We conclude that IL-8 is very stimulatory of keratinocyte growth and has an additive effect on the stimulation of keratinocytes grown with EGF. This effect can be blocked with anti-IL-8, is noted with low dosages ($1x10^{-10}$ g/ml for maximal effect), and thus physiologic importance is predicted.

STIMULATION OF COLLAGEN GENE EXPRESSION BY TGF-ß IN MOUSE DERMAL WOUNDS. George A. Ksander, David R. Olsen, Carolyn O. Gerhardt, Pricilla La Presca, and Hugh McMullin, Celtrix Laboratories, Collagen Corp., Palo Alto, CA

TGF-ß1 and TGF-ß2 have been shown to stimulate transcription of collagen genes in cultured cells and to enhance the healing of dermal wounds. In this study we have examined collagen gene expression in vivo using a mouse full-thickness dermal wound model in the presence and absence of exogenous TGF-ß1 or TGF-ß2. Excisional wounds were created on the dorsum of mice, TGF-ß1 or TGF-ß2 was applied and the wounds were allowed to heal for 7 days. Histomorphometric analysis revealed increased amounts of granulation tissue. RNA was isolated from wound tissue and collagen gene expression was analyzed by Northern blotting. Wounds treated with TGF-ß1 or TGF-ß2 were found to contain elevated levels of α1(I) and α1(III) collagen mRNAs as compared to untreated wounds. The level of tissue inhibitor of metalloproteinases (TIMP-1) mRNA was also increased. These data demonstrate that TGF-ß1 and TGF-ß2 stimulated collagen and TIMP gene expression in healing mouse wounds and this may account for the increases in granulation tissue deposition and enhanced healing.

THE p62 *YES* PROTO-ONCOGENE IS EXPRESSED IN HIGH LEVELS IN BASAL KERATINOCYTES IN NORMAL SKIN AND IS ALTERED IN NEOPLASTIC KERATINOCYTES AND GROWTH-ACTIVATED EPIDERMIS. James Krueger, Yu-Hang Zhao, and Marius Sudol. The Rockefeller University, NY, NY 10021

The *yes* proto-oncogene encodes a 62 kd protein ($p62^{c\text{-}yes}$) which has a protein tyrosine kinase activity related to cytoplasmic domains of growth factor receptors and to other members of the *src*-family of tyrosine kinases. The $p62^{c\text{-}yes}$ kinase may regulate essential early events in cellular growth control since mutants of this protein produce neoplastic cellular transformation in animal models. Specific antibodies to recombinant human $p62^{c\text{-}yes}$ were prepared on affinity columns. Anti-$p62^{c\text{-}yes}$ antibodies showed a strong reaction with the cytoplasmic portion of basal keratinocytes in cryostat sections of neonatal and adult human skin. Staining of basal keratinocytes could be competed with purified *yes* protein. Identity of this immunoreactivity as $p62^{c\text{-}yes}$ was confirmed by specific immunoprecipitation of a 62 kd protein from cultured, radiolabeled normal human keratinocytes and by immunoprecipitation of *yes* kinase activty from unlabeled keratinocyte extracts and from homogenates of human epidermis. Cellular fractionation and immunofluorescence experiments suggest that $p62^{c\text{-}yes}$ is associated predominantly with intracellular membranes in cultured keratinocytes. In growth-activated epidermis from psoriasis and chronic wounds, $p62^{c\text{-}yes}$ was reduced or absent from basal keratinocytes. Nodular basal cell carcinomas showed a reduction in $p62^{c\text{-}yes}$ immunoreactivity compared with basal keratinocytes of normal skin, and a cultured squamous cell carcinoma line (A431) showed a 20-fold reduction in *yes* kinase activity. Expression of $p62^{c\text{-}yes}$ by basal keratinocytes could regulate their proliferation or differentiation in normal and pathological human skin.

RAPID AND SPECIFIC CONVERSION OF INACTIVE PRECURSOR IL-1 BETA TO MATURE ACTIVE IL-1 BY HUMAN MAST CELL CHYMASE: A ROLE FOR MAST CELL PROTEASES IN THE INITIATION OF INFLAMMATORY RESPONSES. T.S. Kupper, N. Schechter, G. Lazarus, and H. Mizutani, Wash. Univ. and U. Penn. Sch. of Med., St. Louis MO and Philadelphia PA.

Many sessile cells in dermis and epidermis can be induced to express mRNA and protein for IL-1 beta, but such cells lack the specific protease (IL-1 convertase) which catalyzes the cleavage of inactive pro-IL-1 beta (33 kD) to the mature (17 mD) form. Thus, IL-1 beta produced by keratinocytes, fibroblasts, and endothelial cells is biologically inactive. 33 kD IL-1 beta must be cleaved between amino acids 110 and 118 to generate an active IL-1 species. Since we have previously shown that chymotrypsin cleaves 33 kD IL-1 beta at Tyr 113-Val 114, we examined other enzymes with chymotryptic specificities produced by cells resident to skin. The addition of highly purified human mast cell chymase to pro-IL-1 beta led to the conversion of the 33 kD protein to an 18 kD protein by Western blot analysis using mAB's to IL-1 beta. The kinetics of the reaction were comparable to those observed with authentic monocyte IL-1 convertase. Sequencing of the converted IL-1 peptide revealed an amino terminus Val 114 and a sequence identical to the chymotryptic product. The biological activity of chymase-generated mature IL-1 was neutralized completely with monospecific anti-IL-1 beta antibodies. We propose that degranulation of mast cells in vivo with attendant release of chymase may serve to activate pro-IL-1 beta produced by convertase negative skin cells, leading to local induction of chemotactic and activating cytokines and adhesion molecules.

ULTRAVIOLET B RADIATION INHIBITS CYTOKINE-INDUCED ICAM-1 GENE TRANSCRIPTION IN HUMAN KERATINOCYTES. Jean Krutmann', Werner Czech', Fritz Parlow', Alexander Kapp', Erwin Schöpf', Thomas A.Luger"·", 'Dept.Derm., Univ. Freiburg, FRG; "LBI-DVS, Lab.Cellbiol., Dept. Derm.II, Univ.Vienna, Austria; "Dept.Derm., Univ.Münster, FRG

Ultraviolet B (UVB) radiation recently has been found to inhibit cytokine-induced ICAM-1 surface expression on transformed (KB, A431) as well as normal human keratinocytes. In order to examine the mechanism responsible for this immunosuppressive activity of UVB light, the effects of UVB radiation on ICAM-1 mRNA expression in human keratinocytes was examined by Northern blot analysis, using an ICAM-1 specific cDNA probe. Unstimulated KB cells contained only low amounts of ICAM-1 specific mRNA, which were significantly increased, if cells were stimulated for 4 hr with IFNγ (500 U/ml). Exposure of KB cells to UVB (100 J/m^2) completely inhibited IFNγ-induced ICAM-1 gene transcription, if cells were irradiated prior to cytokine stimulation. In contrast, UVB irradiation of cells after IFNγ treatment did not affect ICAM-1 mRNA expression. Moreover, sublethal doses of UVB alone (0-150 J/m^2) did not induce ICAM-1 mRNA expression after 4 and 24 hr. After 72 hr, however, an increase in ICAM-1 mRNA expression could be observed. These findings indicate that the UVB induced suppression of cytokine induced ICAM-1 surface expression is due to inhibition of ICAM-1 gene transcription. In addition, UVB may also upregulate ICAM-1 mRNA transcription at a later time point involving not yet investigated mechanisms.

THE EFFECT OF RECOMBINANT BASIC FIBROBLAST GROWTH FACTOR ON THE WOUND HEALING AND ITS LOCALIZATION IN HEALING IMPAIRED DB/DB MICE. Yoriyuki Kurita, Ryoji Tsuboi, Hideoki Ogawa and Daniel B.Rifkin*, Department of Dermatorlogy, Juntendo Univ. School of Medicine, Tokyo, *Department of Cell Biology, New York Univ. Medical Center, New York, NY.

The effect of recombinant basic fibroblast growth factor (bFGF) on wound healing was studied using C57BL/ks J db/db mice. Mice were wounded with a punch biopsy instrument and histological sections of the wounds were evaluated using several histological parameters. Mutant db/db mice showed very poor granulation tissue formation compaired to their normal littermates (db/+). However, the application of 5 µg recombinant bFGF to db/db mice induced significant responses of all of the dermal parameters compaired to non-treated db/db mice ($p<0.001$). Boiled bFGF or bFGF pre-treated with neutralizing antibody lost stimulatory effects. The tensile strength of healed linear wounds in db/db mice was also impaired when compaired to heterozygous littermates. This parameter was also improved by the addition of bFGF to the wounds ($p<0.05$).

Subsequently wound sites were stained with anti-bFGF antibody to show the localization of endogenous bFGF. The basal layer keratinocytes at the wound edge were positively stained, but granulation tissues were not stained 8 days after the wounding.

COMPOSITION OF THE CORNIFIED ENVELOPE OF HUMAN HAIR. Joseph C. Kvedar, Elise B. Bilodeau, Haresh A. Daryanani, and Howard P. Baden, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA.

The cornified envelope (CE) of the hair shaft is located in the cuticle of the cortex. Little is known about the protein composition of this CE and whether precursors to the epidermal envelope contribute to its makeup. Using a panel of monoclonal antibodies to epidermal precursors, we examined the tissue distribution of involucrin, pancornulins, an 82 kD protein, and a 195 kD protein in the human hair follicle. Staining for involucrin was observed in the cytoplasm of the internal root sheath (IRS) and cuticle. Pancornulins were localized there as well. The 82 kD protein localized to the IRS, staining the periphery of cells. The 195 kD protein also localized to the IRS. Monoclonal antibodies were produced from cornified envelopes isolated from a preparation of pure cuticle. These localized to either the outer root sheath, IRS, and/or cuticle. They identified proteins, both tris buffer soluble and 8M urea soluble which were different than the epidermal precursors. These results suggest that some of the epidermal precursors are involved in CE formation in hair and that other previously unrecognized proteins play a role as well.

DEFECTIVE INTERACTION OF A DNA ENDONUCLEASE FROM XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUPS A AND D, CELLS WITH ULTRAVIOLET RADIATION DAMAGED NUCLEOSOMAL DNA. Muriel W. Lambert, David D. Parrish, Gregory J. Tsongalis and Glenn B. Sterling, Department of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ.

We have isolated and partially purified from normal human lymphoblastoid cell chromatin a DNA endonuclease (Endo), pI 7.6, which recognizes pyrimidine dimers created by 254 nm ultraviolet radiation (UVC). This same endonuclease is present in cells from patients with the cancer prone, repair deficient genetic disease, xeroderma pigmentosum (complementation groups A and D) (XPA) (XPD). Utilizing a reconstituted nucleosomal system, consisting of a plasmid DNA and either core (H2A, H2B, H3, H4) or total (H1 + core) histones, we found that the normal Endo was over two fold more active on UVC irradiated core nucleosomal DNA than on non-nucleosomal damaged DNA. Histones H1 reduced this increase. By contrast, the XPA Endo did not show any increase in activity on UVC irradiated nucleosomal DNA and the XPD Endo showed a slight increase, indicating that the XPA and to a lesser extent the XPD Endo are defective in interaction with UVC damaged nucleosomal DNA. Kinetic analysis of the XPA Endo on UVC damaged nucleosomal and non-nucleosomal DNA indicates that the XPA Endo has a reduced affinity for the damaged nucleosomal substrate. Introduction of the normal but not the XPA Endo by electroporation into UVC irradiated XPA cells restored their markedly deficient DNA repair-related unscheduled DNA synthesis (UDS). Furthermore, electroporation of both the normal and XPA Endos into XPD cells complemented the XPD repair defect.

A CALCIUM-SENSITIVE EPITOPE OF THE PEMPHIGUS FOLIACEUS ANTIGEN IS PRESENT ON A MURINE TRYPTIC FRAGMENT AND IS A MAJOR ANTIGENIC DETERMINANT FOR HUMAN AUTOANTIBODIES. RS Labib, B Rock, M Robledo, GJ Anhalt, Department of Dermatology, Johns Hopkins U., Baltimore, MD.

A calcium-sensitive (Ca^{2+}-s) epitope of pemphigus foliaceus antigen (PF Ag) was reported by Eyre and Stanley to be present on the PF complex (4 peptides, 88-260 KD) and to be detected by two thirds of PF sera. Recently, Martins et al reported that a 45 KD tryptic fragment obtained from human skin was specifically immunoprecipitated with all PF sera tested. We have obtained similar results with a murine tryptic fragment of the PF Ag (tf-PF). The present study explores the relevance of the antigenic epitopes on the murine tf-PF to the human autoantibodies (autoAb) and characterizes these epitopes.

PF sera from Brazilian (n=22), Colombian (n=12) and North American (n=5) patients were tested by immunoprecipitation with ^{125}I-labeled murine crude tf-PF preparation in 5 mM $CaCl_2$ and in 5 mM EDTA without Ca^{2+}. All sera immunoprecipitated the 45 KD tf-PF only when Ca^{2+} was present. This data demonstrates that the Ca^{2+}-s epitope(s) is the only epitope detected by all PF sera in the murine tf-PF, and is therefore a major antigenic determinant (MAD).

To investigate the relation of the murine Ca^{2+}-s epitope to all PF Ag epitopes detectable by the human autoAb, we tested 14 PF sera (titers 320- 1280) from Brazilian (n=6), Colombian (n=6) and North American (n=2) patients for blocking of immunofluorescence (IF) on both human (HS) and murine skin (MS) substrates. The IF of 11 sera (1 ul in final volume 100ul) was completely blocked on HS and MS by preincubation with 25 ul of a murine tf-PF preparation. One serum was partially blocked on HS but completely blocked on MS, and two were only partially blocked to both substrates. These results show that less than 25% of PF sera may contain some autoAb that recognize epitopes other than those present on the murine tf-PF, and less than 15% may have autoAb recognizing human-specific epitopes in addition to the common Ca^{2+}-s epitope, confirming that the latter is the MAD. The further characterization of this Ca^{2+}-s MAD will be very important for the understanding of autoimmunity in PF and cell-cell adhesion in the epidermis.

DAMAGE-RESISTANT DNA SYNTHESIS IN XERODERMA PIGMENTOSUM CELLS: REVERSAL FOLLOWING ELECTROPORATION OF NORMAL CHROMATIN-ASSOCIATED ENDONUCLEASES. W. Clark Lambert, Gregory J. Tsongalis, Warren S. Tanz, and Muriel W. Lambert, Departments of Dermatology and Pathology, UMD-New Jersey Medical School, Newark, New Jersey.

Damage-resistant DNA synthesis (DRDS) is a well documented but very rare phenomenon in which replicative DNA synthesis continues at control or near-control rates despite extensive damage to the cells genomic DNA by an agent to which the cells are also hypersensitive. Similarly treated normal cells markedly diminish replicative DNA synthesis. DRDS has been reported in only a very few cell types, including cells derived from patients with ataxia-telangiectasia following X-irradiation, to which they are also markedly hypersensitive, losing viability at several times the normal rate. Xeroderma pigmentosum (XP) is a cancer-prone, repair-deficient genetic disease associated with hypersensitivity to several types of agents, including short wavelength ultraviolet irradiation (UVC) and psoralen plus long wavelength irradiation (PUVA). We now report that XP cells from complementation groups A,C,and D show DRDS as quantitated by ^{3}H-thymidine uptake and autoradiography, following exposure to doses of UVC ranging from 10-80 J/m^2. Treated XP cells show a rate of replicative DNA synthesis several times higher than treated nomal cells. XPA cells exposed to PUVA also show DRDS when compared to normal controls. When either of two normal chromatin-associated endonucleases, pI 4.6 or 7.6, which recognize damage caused by PUVA is electroporated into XPA cells, this DRDS is lost, and replicative DNA synthesis as well as repair-related unscheduled DNA synthesis return to normal levels.

CYTOSKELETAL ELEMENTS INVOLVED IN HUMAN MELANOCYTE DENDRICITY. J.P. Lacour, J. Bhawan, P.R. Gordon, M.Eller, and B.A. Gilchrest, USDA Human Nutrition Research Center at Tufts Univ. and Dept. of Dermatology, Boston Univ. School of Medicine, Boston MA.

Keratinocyte-conditioned medium (KCM) induces stiking dendricity in initially non-dendritic human melanocytes and B16 melanoma cells that is detectable within 30 min, maximal in 24-48 hr, and quantifiable by computerized image analysis. Using the formula dendricity $=1-(4p)(area)(perimeter)^{-2}$, that gives a value of zero for a circle, control cells had a value of 0.32 ± 0.11 (mean $\pm$SEM) while KCM-treated cells had a value of 0.87 ± 0.08. Cytochalasin B (CytoB) 1-10 ug/ml, known to disrupt actin microfilaments, completely blocked dendrite formation (DF) if added before or with KCM; upon refeeding with KCM alone DF occurred normally within 1 hr. In contrast, CytoB treatment had no effect on dendrites induced by KCM $\geq$ 24 hr previously. Agents known to cause microtubule disassembly, colchicine, nocodapole or vinblastine, did not block DF if added individually before or with KCM; but when these agents were added to cells already in KCM for $\geq$ 24 hr, the established dendrites disappeared within 24 hr. Cycloheximide 2-10 ug/ml or actinomycin D 5 ug/ml added 2 hr before and again with KCM completely blocked DF; if cells were placed in fresh KCM lacking these protein synthesis inhibitors, DF resumed after 24 hr. Actin microfilaments visualized with a monoclonal antibody or rhodamine phalloidin were poorly organized in untreated cells but formed numerous elongated fibers in newly KCM-treated cells and were especially prominent along dendrites. These data strongly implicate actin microfilaments in DF but not dendrite maintenance; and conversely microtubules in their maintenance but not formation.

THE AGING EFFECT ON PAPILLARY DERMAL ELASTIC FIBERS IN SUNPROTECTED SKIN BIOPSIES. G Landman*, SK West+, ER Farmer*, Departments of Dermatology* and Ophthalmology+, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

There have been few studies demonstrating the effect of aging on elastic fibers of sunprotected skin. We present the partial results of a longitudinal study attempting to establish a possible relationship between ocular macular degeneration and skin elastic tissue degenerative changes. In a preliminary study, we found absent to minimal changes in the elastic fibers in the reticular dermis using the classification of Kligman. Therefore we studied the elastic changes in the papillary dermis. 394 volunteers were biopsied in the middle aspect of the inner arm, a relatively nonsun-exposed area. The patients were stratified in 10-year cohorts from age 40-years to over 90-years. The biopsies were fixed in buffered 10% formalin and processed for acid-orcein staining. We classified the elastic changes in three grades: grade 0 had no elastic tissue changes (n=170), grade I had minimal (questionable) reduction of oxytalan fibers (n=24), grade II had definite but less than 50% reduction of oxytalan fibers (n=135), and grade III had more than 50% reduction of oxytalan fibers (n=64). A linear regression model controlling for age and sex shows a diminution of oxytalan fibers with increasing age ($p<.001$). At each age, males were more likely to have a greater loss of oxytalan fibers compared to females ($p<.002$). This modified grading scheme is sensitive for age-related changes of fine elastic fibers in sun-protected skin.

REBOUND PHASE OF CORTICOSTEROID-INDUCED CUTANEOUS ATROPHY INVOLVES ANGIOPROLIFERATION AND TUBULORETICULAR INCLUSION FORMATION. Robert M. Lavker, Peishu Zheng, Michael S. Kaminer, and George F. Murphy, Department of Dermatology, Univ. of Pennsylvania, Philadelphia PA.

Cytoplasmic tubuloreticular inclusions (TRI) are observed frequently in endothelial cells and mononuclear cells from patients with AIDS, a condition also linked to clinical and subclinical angioproliferation. In human tissues, (TRI) are composed of loose collections of tubules, whereas in animal tissues they have a paracrystalline appearance. Ultrastructural studies of hairless micropig (HMP) dermal microvasculature during proliferative recovery (rebound) from experimentally-produced corticosteroid atrophy (J.I.D. 90:574,1988) demonstrated numerous paracrystalline inclusions in endothelial cells. Affected vessels showed increased profiles characterized by dilated, interconnecting channels, often with jagged, irregular contours. TRI were never observed in control HMP skin or during 6 weeks of steroid treatment. Inclusions were non-membrane-bound, displayed a highly ordered periodic structure similar to animal TRI, and appeared as early as 2 days after discontinuation of steroid treatment. Whereas endothelium immediately prior to withdrawal failed to demonstrate tritiated thymidine incorporation or Factor VIII immunoreactivity, return of these markers of proliferative and biosynthetic activity was coincident with TRI expression. Paracrystalline inclusions were not observed in endothelial cells during the angioproliferative response resulting from experimentally-induced incisional wounds.

Previous studies using this model indicate that steroid treatment alters the normal cytokine-rich microenvironment (J.I.D. 92:411, 1989). Thus, angioproliferation and TRI expression as a result of steroid rebound are consistent with the concept that microvascular homeostasis may be in part regulated by cytokines. This model also may facilitate understanding of potential associations between impaired local immunity, angioproliferation, and TRI formation, as is commonly encountered in AIDS.

ROLE OF PROTEIN KINASE C IN SKIN MICROVESSEL ANGIOGENESIS. J. Lee and M. Karasek. Dept. Dermatology, Stanford University, Stanford, CA

Angiogenesis plays a central role in wound healing, tumor growth, and inflammatory skin diseases. Although several angiogenic factors have been isolated and characterized, there is little information on the mechanisms by which these factors control new vessel growth. In this study we have examined the role of activators of protein kinase C in microvascular angiogenesis and show that this enzyme plays a central role in regulating new vessel growth in in vitro models.

Microvascular endothelial cells were isolated from newborn foreskin and grown in a modified Iscove's medium. Angiogenesis was induced in monolayer cultures by exposure of the apical surface of the culture to a collagen gel. Under these conditions, endothelial cells reorganize into vessel-like structures. Phorbol myristate acetate (PMA) (10 ug/ml) was used to stimulate and 1-(5-isoquinolinysulfonyl)-2-methyl piperazine (H-7) (10^{-6} M) to inhibit protein kinase C. Prior to stimulation with PMA, cells were pulsed with ^{32}phosphate, and phosphorylated proteins were analyzed by two dimensional PAGE.

PMA caused an increase in the rate and extent of new vessel growth. Addition of H-7 prior to PMA completely inhibited vessel formation and cells remained in a typical epithelioid monolayer. Removal of H-7 resulted in an immediate reorganization of the cells into vessels. Phosphate incorporation into soluble proteins was stimulated by TPA, and the incorporation was completely blocked by H-7.

These results demonstrate a close relationship between activation of protein kinase C, intracellular protein phosphorylation and new vessel formation. The ability to inhibit angiogenesis by inhibiting protein kinases may provide a new pharmacologic approach for the control of abnormal skin blood vessel growth.

CONTAMINATION OF INJECTABLE BOVINE COLLAGEN NEEDLE TIPS WITH PROPIONIBACTERIUM ACNES. Mark Lebwohl, Ed Bottone, Marsha Gordon, and Dolores Lucas, Departments of Dermatology and Microbiology, Mt. Sinai Medical Center, New York, N.Y.

We microbiologically examined injectable bovine collagen (IBC) remaining in syringes stored after use in human subjects. IBC has been used for the correction of cutaneous depressions in over 350,000 people. Because of IBC's expense and the small amounts required for correction of limited defects, some practitioners reinject IBC from partially used syringes even after months of storage. Reports of sterile abscess formation at implantation sites, nevertheless, are rare.

50 syringes of IBC were capped after partial use and stored at 4°C for up to 38 months. Samples were inoculated directly in thioglycolate broth and needle tips were inoculated separately. Of needle tip cultures, 4 grew P. acnes, 1 Staphylococcus epidermidis, and 1 S. aureus. Only one collagen culture grew a non-hemolytic streptococcus.

We conclude that infection with P. acnes can occur following IBC implantation as a consequence of contaminated needle tips. Changing the needle tips is likely to reduce the chance of infection. Because P. acnes may grow slowly and only under anaerobic conditions, this organism may be the etiologic agent responsible for injectable collagen-related abscesses that are thought to be sterile.

RNA Metabolism in Differentiating Keratinocytes in Culture.

Joseph I. Lee, Chana Fuchs, and Lorne B. Taichman. Dept. of Oral Biology and Pathology, SUNY, Stony Brook, NY.

Steady state levels of mRNA for certain genes (eg, involucrin) change during keratinocyte differentiation. Steady state levels of mRNA reflect a balance between rate of transcription and rate of degradation. We have examined synthesis and degradation of several gene transcripts in cultured foreskin keratinocytes during terminal differentiation.

The specific genes examined were involucrin, glyceraldehyde phosphate dehydrogenase, N-, K- and H-ras and apolipoprotein E. Keratinocytes were centrifuged in Ficoll400 gradients and measurements were performed in fractions enriched for small and large cells. Steady state levels of involucrin mRNA, as measured by northern hybridization, increased in large cells but levels of other gene transcripts did not change. Rates of transcription, as measured by nuclear run-off assay, increased for involucrin but not for the other genes. Involucrin mRNA half-life as measured by decay was the same in both large and small cells. With the data at hand we conclude that increases in involucrin mRNA in differentiating keratinocytes result from increased rate of transcription. These studies provide a baseline for exploring transcriptional and post-transcriptional gene regulation in differentiating keratinocytes.

UV-INDUCED POTENTIATION OF HISTAMINE-STIMULATED PROSTAGLANDIN SYNTHESIS OCCURS THROUGH AN INCREASE IN PROTEIN KINASE C. K. Lee Knutzen Steuer and Alice P. Pentland, Div. of Dermatology, Washington University, St. Louis, MO 63110.

Ultraviolet light injury to skin is manifested acutely as erythema and is mediated, in part, by histamine-stimulated prostaglandin (PG) synthesis. The signal transduction mechanism of this enhanced PG synthesis has not been determined. To evaluate the role of protein kinase C (PKC), PKC was partially purified from adult human epidermal cultures and assayed by measuring incorporation of ^{32}P-ATP into histone. Cultures irradiated with 30 mj/cm^2 UV using Westinghouse FS20 lamps contained twice the quantity of cytosolic PKC relative to unirradiated cultures. After histamine stimulation, cytosolic PKC decreased to comparable levels in control and irradiated cultures, suggesting enzyme translocation to the membrane. To determine if this increase in PKC mediated enhanced histamine responsiveness, keratinocytes were treated for 72 hours with TPA to eliminate PKC activity by down-regulation. In TPA pretreated cultures, UV no longer enhanced histamine-stimulated PG synthesis. Therefore, our preliminary data suggests that UVB increases PKC in keratinocytes. This UV-stimulated increase in PKC mediates enhanced PG synthesis in response to histamine.

THE 50 KD LA PROTEIN IS PRESENT IN GREATER AMOUNTS IN NEONATAL V. ADULT TISSUES. Lela A. Lee and Kristen B. Rundell. Depts. of Dermatology and Medicine, University of Colorado School of Medicine and Denver VA, Denver, CO.

Neonatal lupus (NLE) is an autoimmune disease associated with maternal anti-Ro/SSA and anti-La/SSB autoantibodies. Babies with NLE generally have heart or skin disease, while their autoantibody-producing mothers are asymptomatic or mildly symptomatic. The reason for this discordance between symptoms in babies and mothers is not known. NLE sera react in immunoblotting with 60 kDa Ro/SSA protein and/or with 50 and 45 kDa proteins present in extracts of human tissue. It has recently been observed that the 50 kDa protein is present in increased amounts in neonatal v. adult tissues. Its molecular weight is consistent with its being La/SSB, a ribonucleoprotein that has a role in RNA processing. La protein is 50 kDa and reportedly degrades readily to 45 kDa. In order to define further the 50 kDa protein reactive with NLE sera, the following studies were done:

Human spleen extract was run in SDS-PAGE. The 50 & 45 kDa regions were cut out of the gel, loaded onto another polyacrylamide gel, transferred to nitrocellulose, and immunoblotted with NLE serum. When the 50 kDa region was cut out, rerun, and blotted, bands were identified at 50 and 45 kDa, consistent with the 50 kDa proteins' being La with a 45 kDa breakdown product. The rerun 45 kDa region had only a band at 45 kDa. Second, NLE serum was adsorbed to a La affinity column. The adsorption resulted in absence of reactivity of the serum with both the 50 & the 45 kDa regions. When the antibodies bound to the affinity column were eluted from the column, the eluted antibodies reacted with 50 & 45 kDa regions on immunoblotting. Finally, human spleen extract was run in 2-dimensional gel electrophoresis and immunoblotted. The 50 kDa region was resolved into 2 bands that may represent charge isomers of La.

These studies indicate that the protein that has been described as being present in greater amounts in neonatal tissues is the RNA processing ribonucleoprotein, La. It is possible that its abundance in neonatal v. adult tissues is related to the association of anti-La antibodies with autoimmune neonatal disease.

IMIDAZOLES AND NEUTROPHIL FUNCTION. W. Lee and A. Shalita, Dept. of Dermatology, SUNY Health Science Center at Bklyn, Bklyn, N.Y.

Imidazoles are a group of antimycotic drugs with activity against a wide range of pathogenic fungi, particularly dermatophytes. Clinically, many of these compounds also appear to have anti-inflammatory activity. Since neutrophils (PMN) play an important role in cutaneous inflammation, we wish to assess the in vitro effect of these drugs on inflammatory cells by the measurement of PMN chemotaxis (CT) and chemiluminescence (CL).

Stimulation of PMN, preincubated with the imidazole (37°C for 30' at 1-250 ug/ml), by the chemoattractants f-met-leu-phe, latex and zymosan showed a dose-dependent inhibition in PMN CT and CL; the CT response was reduced by 95% at 60 ug/ml of terconazole(TC) whereas the CL response (emission of photons from reactive oxygen species, ROS, generated during membrane activation) showed one log reduction at 5-15 ug/ml. The mechanism for imidazole inhibition of PMN function is not due primarily to cell death or metabolic inactivation as demonstrated by experiments with trypan blue exclusion and bacterial killing of Staphylococcus aureus. Our data showed that TC was the most active product, followed by miconazole and ketoconazole, whereas isoconazole and itraconazole were the least active compounds under our experimental condition.

It is postulated that the capacity of these agents to inhibit PMN migration and ROS production may account, in part, for their efficacy in inflammatory skin diseases.

TRICHILEMMOMAS ARE NOT ASSOCIATED WITH HUMAN PAPILOMMA VIRUS DNA. C.L. Leonardi,* W.Y. Zhu,* N.S. Penneys* and W.H. Kinsey,± Departments of Dermatology and Cutaneous Surgery* and Department of Cell Biology and Anatomy,± University of Miami School of Medicine, Miami, Florida.

Trichilemmomas are benign tumors arising from the outer root sheath of the hair follicle. A relationship between Human Papilloma Virus (HPV) infection and the development of these tumors has been suggested based on morphologic observations. We have analyzed 25 specimens for the presence of HPV using DNA amplification technology. Briefly, DNA from each specimen was extracted from four 10um sections of paraffin-embedded tissue diagnosed as trichilemmoma. A set of oligonucleotide primers were used which amplify a highly conserved region of the HPV L1 open reading frame. We were unable to detect the 450 BP amplimer that is characteristic of HPV by electrophoresis, or slot blot hybridization with consensus and type specific probes (HPV's 6, 11, 16, 18, 33). Amplification controls (specimen and internal) were positive. These results do not support the concept that occult HPV infection is involved with the development of trichilemmoma.

PERCUTANEOUS ABSORPTION OF RETINOL, RETINOIC ACID, RETINYL ACETATE, AND RETINYL PALMITATE THROUGH HUMAN STRATUM CORNEUM IN-VITRO. Paul A. Lehman and Thomas J. Franz, Division of Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Because of regulated prescription limitations on the use of topical retinoic acid (Retin-A) there is now renewed interest in topical formulations of Vitamin A (retinol) and its ester analogs. As percutaneous absorption precedes the question of efficacy, we investigated the permeability of retinol (ROL), retinyl acetate (RAC) and retinyl palmitate (RP) through human stratum corneum in-vitro and compared them to topical all-trans retinoic acid (ATRA).

After acquiring epidermal preparations of autopsy skin from 4 donors by heat separation stratum corneum was isolated by trypsin digest of the viable cell layers and was mounted onto Franz diffusion chambers which contained saline/0.5% Volpo receiver solution. Individual retinoids (0.035M ROL, RAC, RAP, and 0.002M ATRA), in a base vehicle (water-in-oil), were topically applied (20 ul/cm^2) to the stratum corneum and removed by surface wash 24 hr later. Retinoid penetration into the receiver solution over 24 hr, surface wash and stratum corneum content was quantified by HPLC.

ROL demonstrated the greatest penetration at 0.97% of the applied dose followed by ATRA (0.76%), RAC (0.15%) and RP (0.04%). Stratum corneum content at 24 hr was greatest for ATRA (1.43%) followed by RP (0.58%), ROL (0.13%) and RAC (0.12%).

In addition some inter-conversion of retinoids was also observed. In the surface wash ROL was found on skins to which RAC and RP was applied, and ATRA was found on skins to which RAC was applied. In the receiver solutions ATRA and RP were found from skins to which ROL was applied. This observation suggests that stratum corneum may retain some enzymatic activity pertinent to retinoid metabolism and storage.

MOLECULAR DIAGNOSIS OF CUTANEOUS T-CELL LYMPHOMA (CTCL): DEVELOPMENT OF A MOLECULAR PROBE SPECIFIC FOR A PATIENT'S MALIGNANT T-LYMPHOCYTES. SR Lessin'·*, AH Rook*, G Rovera*. Dept. of Dermatol., Univ. of Penna.', The Wistar Institute*, Phila., Pa. USA.

The goal of this study was to identify the specific V, D, J & C segments utilized in T-cell antigen receptor (TCR) β-chain gene rearrangement from malignant T-cells of a patient with CTCL (Sezary syndrome) in order to generate a specific oligonucleotide probe to detect CTCL cells through polymerase chain reaction (PCR) amplification.

Total RNA isolated from peripheral blood lymphocytes was reversed transcribed with a primer to the TCR-β constant (C) region. The resultant cDNA was PCR amplified utilizing a 5'primer consisting of consensus sequences to a highly conserved region in the V_β gene region and a 3'primer to C_β sequences. PCR reaction products were subcloned into a plasmid vector and sequenced.

Sequence analysis revealed that the patient utilized V_β-6.4, D_β-1.1, J_β-2.2 and C_β-2 gene segments. Oligo primers to V_β-6.4 and J_β-2.2 were utilized to PCR amplify genomic DNA taken from patient's blood and involved skin. Screening the amplified DNA with a oligo probe specific for the patient's V-D-J-junctional sequences resulted in detection of patient specific sequences. No sequences were detected from DNAs from other CTCLs or benign infiltrates. Thus, we have defined a molecular "fingerprint" specific for a patient's malignant T-cells and can molecularly detect CTCL through PCR amplification.

PERCUTANEOUS ABSORPTION OF SALICYLIC ACID: ASSESSMENT OF RELATIVE BIOEQUIVALENCE OF FOUR COMMERCIALLY AVAILABLE TOPICAL FORMULATIONS IN-VITRO. Paul A. Lehman and Thomas J. Franz, Div. of Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Salicylic acid (SA) is frequently used as a mild topical keratolytic agent. The objective of this study was to determine if different commercial formulations of salicylic acid demonstrate equivalent bioavailability, as assessed by its percutaneous absorption in-vitro. The products tested were two different solution vehicles (Duoplant and Occlusol-HP) and two different transdermal patch systems (Transplantar and Dr Scholls).

Back skin, dermatomed from cadaver donors, was mounted onto Franz diffusion chambers and received 20 ul/cm^2 applications of either vehicle or the direct application of either transdermal patch. In addition, the two solution vehicles were also examined on plantar skin from a single donor. Penetration was determined by monitoring SA in receiver solution samples over the 24 hr topical exposure period by high pressure liquid chromatography.

The percutaneous absorption of SA from the two solution vehicles was not significantly different. Their penetration flux profile was characterized by a classic rapid rise and fall curve shape showing a peak flux of 500 $ug/cm^2/hr$ at 1.5-2 hr and total penetration of appx 3000 ug. On plantar skin the curve profile was characteristic of a thicker membrane with a peak flux of 200 $ug/cm^2/hr$ at 3-4 hrs.

In contrast, the two transdermal patch systems demonstrated different percutaneous absorption kinetics. The flux profile from Dr. Scholls patch was essentially steady-state at 45 $ug/cm^2/hr$ throughout the 24 hr exposure period with 1040 ug total penetration. The flux profile of the Transplantar patch steadily rose over the application exposure period achieving 200 $ug/cm^2/hr$ only at 24 hr with 3000 ug total penetration of salicylic acid.

COMPARISON OF LASER-INDUCED PHOTOTOXIC PROPERTIES OF ALUMINUM PHTHALOCYANINE DISULFONATE AND TETRASULFONATE ON HUMAN CELLS IN VITRO. L. Lewandowski, E. Glassberg, G. Galler-Rimm, G. Lask and J. Uitto. Thomas Jefferson University, Philadelphia, PA.

Metallo-phthalocyanine dyes can be used to preferentially sensitize and destroy malignant cells or tissues when exposed to activating light energy. Aluminum phthalocyanine disulfonate (DS) and tetrasulfonate (TS) are structurally related photosensitizing compounds which demonstrate markedly different degrees of hydrophobicity, which, in turn, affects their passive transport into cells. In this study, we compared the photosensitizing efficiency and neoplastic cell-selectivity for these dyes in cultures of human HT1080 fibrosarcoma cells and normal fibroblasts. After a 16-hr incubation with either DS or TS, cultured cells were exposed to laser energy at 675 nm (the absorption peak of both dyes) and assayed for cytotoxicity using [^{3}H]thymidine and [^{35}S]methionine incorporation as markers for DNA and protein synthesis, respectively. In both cell lines, DS and TS (3 or 10 $\mu g/ml$) elicited an energy density-dependent toxicity. However, cells incubated with TS required an ~4-fold higher energy density to achieve a comparable level of toxicity as noted with DS. However, under conditions where TS showed maximal anti-neoplastic cell activity, DS demonstrated only minimal to moderate selectivity. Single-cell fluorescence microscopy showed markedly greater accumulation of DS in cells when compared to TS. Thus, while DS may be a more efficient photosensitizer than TS, it appears to be less selective towards neoplastic cells, possibly because of its greater ability to penetrate both normal and malignant cells.

CHARACTERIZATION OF PUNGENT AXILLARY ODORS. J. J. Leyden, X.-N. Zeng, K. McGinley, H. J. Lawley and G. Preti, Department of Dermatology, University of Pennsylvania and Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

Previous studies of axillary odors have suggested that volatile steroids and isovaleric acid are major contributors to the pungent odor produced in the underarm region. Acid-base extractions of axillary secretions collected on cotton pads followed by organoleptic evaluation of gas chromatographic separated components of the extract was employed to isolate the characteristic underarm odors. Combined gas chromatography/mass spectrometry (GC/MS) as well as gas chromatography/Four ier Transformer Infrared Spectrometry showed that characteristic axillary odor consisted of C_6 to C_{10} normal, unsaturated and branched chained acids with the most abundant being trans-3-methyl-2-hexenoic acid. Apocrine secretion was obtained from several volunteers by intracutaneous injection of epinephrine. Partitioning this apocrine secretion into aqueous and organic soluble fractions was followed by hydrolysis with methanolic NaOH. This process yielded a strong characteristic axillary odor from the acidified aqueous hydrolysate. No odor was found in the acidified organic fraction. Analysis by GC/MS revealed the presence, in the aqueous hydrolysate, of the C_6 to C_{10} acids, including trans-3-methyl-2-hexenoic acid. These results suggest water soluble precursor(s) are responsible for pungent axillary odors.

EFFECTS OF T CELL FACTORS ON MONOCYTE PHOSPHODIESTERASE ACTIVITY. Shi Hua Li, Sai C. Chan, Donald Y.M Leung and Jon M. Hanifin. Dept. of Dermatology, Ore. Health Sci. U., Portland, Oregon and Dept. of Pediatric Allergy and Immunology, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado.

Patients with atopic dermatitis (AD) have increased monocyte cyclic AMP phosphodiesterase (PDE) activity compared to normal (NL) subjects. Because of evidence for increased expression of interleukin-4 (IL-4) by T cells from patients with AD, we asked whether elevated PDE activity in AD results from the products of abnormal T cell activation.

Monocytes were isolated from Histopaque-separated peripheral blood mononuclear leukocytes by adherence. T-lymphocytes were further purified from non-adherent cells by rosetting with sheep red blood cells. Normal monocytes were incubated for 60 minutes with 24 hour pooled T cell culture supernatants (Sups) from AD and NL donors. The monocytes were then radioenzymatically assayed for PDE activity.

Both AD and NL Sups significantly stimulated NL monocyte PDE. AD Sups increased monocyte PDE activity up to 390%, but this increase was not significantly greater than that caused by paired NL Sups ($P=0.58$, $N=4$). Recombinant human (*rh*) IL-4 also significantly increased NL monocyte PDE activity to a maxium of 188% at 50 units/ml ($P<0.05$, $N=6$). The effect of both *rh*IL-4 and AD T cell Sups on NL monocytes was neutralized by a polyclonal antibody against *rh*IL-4. Though interferon-γ (IFN-γ) blocked some of IL-4 effects, we found that IFN-γ increased monocyte PDE activity 310% ($P<0.05$, $N=3$), and the effects were blocked by anti-IFN-γ.

We found no evidence for excessive AD T cell stimulation of moncyte PDE activity. The study demonstrates that T cell factors such as IFN-γ and IL-4 can increase PDE activity in normal moncytes. This finding may indicate a common mechanism for lymphokine modulation of the cyclic nucleotide regulatory pathway.

THE ANTIBODY RESPONSE TO IMMUNIZATION TO A POLYVALENT MELANOMA ANTIGEN VACCINE. J Li, M Henn, R Oratz, J-C Bystryn, Dept of Dermatology and Kaplan Cancer Center, NYU School of Medicine, New York, NY.

We characterized the antibody response induced by a polyvalent melanoma antigen vaccine in patients with early melanoma. Twenty six sequential patients with post-surgical stage II melanoma were immunized to 40 ug of the vaccine q3wks x 4. Melanoma antibodies were measured by an improved immunoprecipitation assay in which serum is incubated with soluble radioiodinated melanoma surface macromolecules, bound antigens (ags) are precipitated with protein-A sepharose, and analyzed by SDS-PAGE and autoradiography. Antibodies to human melanoma were present in 3 (11%) pts prior to immunization and in 19 (73%) pts after the 4th immunization. The antibodies present prior to immunization were directed predominantly to ags expressed by both melanoma and control cells. The vaccine induced or augmented antibody responses to one or more melanoma cell surface ags with approximate MWs of 38, 75, 110, 150 or 200+ kDs. Antibodies were induced most often to the 110 and 200+ kD ags (in 62% and 27% of pts) and then to the 38, 150 and 75 kds antigens (induced in 19%, 8% and 8% of pts respectively). The 200+ kD and 110 kD antigens were preferentially expressed on melanoma as both were present on 4 of 5 human melanoma cell lines but on only 2 of 9 control cells.

These results indicate that melanoma vaccine immunization stimulates antibody responses to melanoma in over 60% of pts and that the antibodies are directed to various combinations of melanoma cell surface antigens. The most immunogenic of these antigens have approximate MWs of 110 and 200+ kds.

ANTI-5α-REDUCTASE AUTOANTIBODIES IN THE SERUM OF PATIENTS WITH PROSTATIC CANCER. Tehming Liang and Shutsung Liao, Section of Dermatology, Department of Medicine, Ben May Institute, and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL.

5α-Reductase converts testosterone to a more active androgen, 5α-dihydrotestosterone that binds to a nuclear receptor in target cells. We have investigated whether anti-5α-reductase autoantibodies are present in human serum.

Serum samples were tested for their ability to inhibit binding of a potent radioactive 5α-reductase inhibitor ([^{3}H]4-MA, Endocrinology, vol. 117, 571-579, 1985) to 5α-reductase and to inhibit the reductase activity in rat liver microsomes. Only 4 serum samples from 81 prostate cancer patients showed inhibitory activity, whereas 158 other adult male serum samples were not active. Immunoglobulins (IgG) were purified from the 4 inhibitory serum samples by protein A affinity column chromatography. More than 90% of the inhibitory activity was found in the IgG fractions. The inhibitory activity of these IgG preparations could also be demonstrated with 5α-reductase preparations solubilized from microsomes and partially purified by DEAE-Sepharose column chromatography. There was no inhibitory activity in the IgG fractions purified from 4 non-inhibitory serum samples. The inhibitory activity was apparently not due to steroids or heavy metal ions in the active serum. These results suggest the presence of autoantibodies to 5α-reductase in some of prostate cancer patients. Autoantibodies may be useful in the study of the role of 5α-reductase in androgen-dependent disorders, such as acne, male pattern baldness, hirsutism, benign prostatic hyperplasia and prostatic cancer.

THE DIFFERENTIAL EFFECTS OF TRANSFORMING GROWTH FACTOR-β (TGF-β) ON INTERFERON-γ-INDUCED EXPRESSION OF INTERCELLULAR ADHESION MOLECULE (ICAM)-1 AND DRβ IN HUMAN KERATINOCYTES (HK). L-J. Li, K. Degitz, S.W. Caughman, Derm Branch, NCI, NIH, Bethesda, MD.

De novo expression of both ICAM-1 and DRβ by HK has been demonstrated in various inflammatory conditions and in response to various cytokines, including interferon (IFN)-γ. TGF-β has been previously demonstrated to inhibit IFN-γ-induced upregulation of DR in certain cell lines and implicated in suppressing ICAM-1 expression by endothelial cells. Since both IFN-γ and TGF-β are commonly secreted in cutaneous inflammation, the effects of TGF-β upon IFN-γ-induced ICAM-1 and DRβ mRNA and cell surface expression by HK was determined by northern blot analysis and flow cytometry. The expression of both ICAM-1 and DRβ by HK in response to IFN-γ is transcriptionally regulated, but the kinetics of their induction differ markedly: mRNA and surface expression of ICAM-1 are induced as early as 3 and 6 hr, respectively, after IFN-γ (250 U/ml) treatment, while similar DRβ expression could not be detected until 18 and 24 hr. Treatment of HK with TGF-β (5 ng/ml) alone did not induce either ICAM-1 or DRβ. TGF-β pretreatment of HK had opposite effects upon IFN-γ-induced ICAM-1 and DRβ expression in that ICAM-1 expression was enhanced, while DRβ expression was suppressed. ICAM-1 expression was enhanced when TGF-β was added for as little as 1 hr before a 3 hr IFN-γ-induction; DRβ expression was suppressed even when TGF-β was added as long as 3 hr after beginning IFN-γ-induction. Cycloheximide pretreatment before addition of IFN-γ and/or TGF-β revealed that while de novo protein synthesis was not required for the induction of either ICAM-1 or DRβ mRNA by IFN-γ alone, it was required for the observed effects of TGF-β. These data indicate that both IFN-γ and TGF-β are important in the regulated expression of ICAM-1 and DRβ in HK, that TGF-β differentially modulates the IFN-γ-induced expression of ICAM-1 and DRβ, and suggest that this modulation requires de novo synthesis of TGF-β-induced protein factor(s) which can either enhance or suppress gene expression.

SUCCESSFUL TRANSFER OF ORGANIZED GRANULOMAS FROM MURINE SKIN. T.Iida, N. Sato, Y. Nozaki, Y. Sasaki and W.L. Epstein, Department of Dermatology, University of California, San Francisco, CA.

We investigated morphological and biochemical changes which associate with granuloma (Gr) development in murine skin caused by inoculation of Gr components not containing infectious substances or live donor cells. Skin Gr after transplantation of freeze-dried hepatic schistosome egg Gr were excised, freeze-dried for a second time, and grafted in C57BL/6 mouse skin. Light microscopy showed accumulation of mononuclear cells around the inoculum from 1 wk after grafting, but Gr did not develop up to 4 wks. By 5 wks organized Gr began to develop around capillaries, destroyed parasite eggs and other unidentified partcles. By electron microscopy activated macrophages and epithelioid cells were observed. Angiotensin converting enzyme (ACE) activity (mU/mg protein) was measured using hippuryl-His-Leu as a substrate. ACE activity in grafts from donor mice disappeared within 1 wk, but began to elevate in the lesion by 4 wks and significantly ($p<0.01$) increased by 5 wks.

	Grafted Gr	1wk	2wks	4wks	5wks
ACE	563.5±42.3	226.0±7.0	228.7±13.4	281.8±6.6	516.2±12.7

Injection of cyclosporine (150 mg/kg/day; 5 day/wk for 5 wks) did not affect Gr formation. These results indicate that organized Gr resembling sarcoid Gr can be elicitated by Gr component without T cell participation. The effects of the inoculum differs from a positive Kveim reaction which is seen only in patients with sarcoidosis, and not in normal individuals.

VARIANT EPITOPE BINDING PROFILE (EBP) OF DRUG-INDUCED Ro/SS-A AUTOANTIBODIES (a-Ro). T-S Lieu, D.P. McCauliffe, L.A. Lee, D.A. Norris, J.D. Capra, R.D. Sontheimer. Depts. of Dermatology and Microbiology, U.T. Southwestern Med. Center, Dallas, TX and Dept. of Dermatology, University of Colorado School of Medicine, Denver, CO.

We have questioned whether the a-Ro produced by drug (e.g. hydrochlorothiazide)-induced (D-I) SCLE pts have the same EBP as do a-Ro produced by idiopathic (ID) SCLE pts and mothers of infants with congenital heart block (Mat. CHB). Six synthetic peptides (pptd) were prepared corresponding to portions of the deduced amino acid (AA) sequence of an acidic, calcium-binding 46 kD human Ro protein which migrates aberrantly at 60 kD in SDS-PAGE. IgG reactivity against these pptd present in sera having similar Ro precipitin levels was determined by enzyme-linked immunosorbent analysis (ELISA). The % of elevated optical density (OD) values are shown in the table:

	Ro synthetic pptd (AA position numbers)					
Pt group	7-24	88-103	171-193	184-194	186-203	382-400
D-I SCLE (n=11)	36%	36%	36%	18%	36%	27%
ID SCLE (n=28)	82%	71%	82%	46%	89%	82%
Mat. CHB (n=10)	90%	30%	90%	40%	90%	70%

In addition to a lower overall frequency of synthetic pptd binding, the D-I SCLE sera also generally produced lower ELISA OD readings. These findings suggest that the a-Ro present in D-I SCLE sera have a variant EPB for a 46(60) kD Ro autoantigen compared to the a-Ro produced by ID SCLE pts and Mat. CHB. and raise the possibility that the mechanism responsible for a-Ro production in D-I SCLE might differ from those triggering a-Ro synthesis in ID SCLE and Mat. CHB.

ISOLATION OF A HUMAN EPIDERMAL cDNA ENCODING A FUSION PROTEIN RECOGNIZED BY PEMPHIGUS FOLIACEUS SERA. Z. Liu, L. A. Diaz and G. J. Giudice. Dept. of Dermatology, Med. College of Wisconsin, Milwaukee, WI.

One third of the sera of patients with Pemphigus Foliaceus (PF) recognize a 160 kD epidermal protein identified as Desmoglein I (DG I) by immunoblot analysis (J.Invest Derm. 87:197, 1986).

In this study we have screened a lambda gt11 human keratinocyte cDNA library with a well- characterized PF serum containing IgG4 PF autoantibodies. One immunoreactive clone was isolated and subjected to further immunological analyses. A lysogen was generated by infecting Y1089 E. coli with the isolated recombinant bacteriophage and was maintained in liquid culture at 30°C. The cDNA-encoded fusion protein was induced by increasing the temperature to 42°C in the presence of IPTG. The bacterial protein extract was then analyzed by immunoblot analysis using PF and control sera. The 140 kD beta galactosidase fusion protein was recognized by PF sera (8 of 25), by IgG4 PF autoantibodies and by an anti-DG I serum (M.S. Steinberg, Princeton Univ.), but not by any of the control sera including bullous pemphigoid (n:5) and normal human sera (n:10). These findings suggest that the recombinant fusion protein which was identified on the basis of its reactivity with a PF serum may share antigenic determinants with human epidermal DG I.

UVB IRRADIATION OF KERATINOCYTES INDUCES GENERATION OF EICOSANOIDS FROM ENDOTHELIAL CELLS. Henry W. Lim, Elizabeth Baker-Owens, and Mahesh Sarmalkar, Dermatology Service, New York VA Medical Center, and Department of Dermatology, New York University School of Medicine, New York, NY.

This study was designed to investigate keratinocyte-endothelial cell interactions in ultraviolet (UV)-induced inflammation. Cultured human keratinocytes (KC) were irradiated with 5 kJ/m² of UVB; at 5 min, 2,4,5,15,18 and 24 hrs after completion of irradiation, KC supernatants were incubated for 1 hr with ³H-arachidonic acid-labeled human umbilical vein endothelial cells (EC). Incubation of EC with supernatants obtained at 4 and 5 hrs resulted in net release of radioactivity of 8.5 ± 1.2% and 11.3 ± 0.5%, respectively; no release was noted at other time-points studied. Radio-thin layer chromatographic analysis of the eicosanoids released showed that 6-keto-PGF1a and PGF2a were the predominant products generated. Eicosanoid generation was suppressed by incubation of KC supernatants with anti-interleukin (IL)-1-a, but not by anti-IL6, antibodies in an antibody dose-dependent fashion. Release of radioactivity was suppressed by 60% when KC were incubated with dexamethasone (10^{-5}M) prior to UVB exposure, while preincubation of KC with 1mM aspirin, a cyclooxygenase inhibitor, had no effect. These data demonstrate that in UVB-induced inflammation, KC-EC interactions do occur, which are mediated, at least in part, by KC-derived IL1.

EXPRESSION OF TRANSFECTED CD1a: DEVELOPMENT OF AN IN VITRO IMMUNOPHENOTYPIC MODEL OF HUMAN EPIDERMAL LANGERHANS CELLS (LC). Jack Longley, Tie Gang Ding, Paula Kavathas, and Richard Edelson, the Departments of Dermatology and Laboratory Medicine, Yale University, School of Medicine, New Haven, CT.

In order to develop stable in vitro models of human epidermal LC, cell lines were transfected with an expression vector containing a copy DNA encoding the human LC surface molecule CD1a. One of these cell lines is based on a human lymphoblastoid cell line called 221 which expresses its own class II major histocompatibility (MHC) molecules but is deficient in class I MHC molecule expression. The resulting cell line, 221CD1a, has a surface phenotype similar to epidermal LC in that it expresses CD1a and class II MHC molecules at levels comparable to freshly isolated human LC. In addition to lymphoblastoid cell lines, murine L-cells were co-transfected with the CD1a expression vector and with expression vectors encoding the alpha chain of human class II MHC molecule (HLA-DR) and the DrBeta 1 allele from a patient with the DR7 haplotype. The resulting cell line, L-CD1aDrB7, expresses both CD1a and HLA-Dr7 and thus demonstrates it is possible to custom design cell lines that have an immunophenotype at least partially matching that of the epidermal LC of a specific individual.

Since human lymphblastoid cell lines are capable of presenting soluble antigen in vitro to T-cells, these cell lines represent valuable tools for investigating the role of CD1a in antigen presentation and may allow antigen specific stimulation of an individual patient's T-cells.

RETICULOENDOTHELIAL PROPERTIES OF SKIN MICROVESSEL ENDOTHELIAL CELLS (MEC). B. H. Lipton, K. G. Bensch, and M. A. Karasek. Departments of Dermatology and Pathology, Stanford University, Stanford, CA

Under homeostatic conditions cultured MEC characteristically exhibit an epithelial configuration with zonular junctions, a non-thrombogenic luminal surface, Weibel/Palade bodies and Factor VIII antigen. However inflammatory perturbations profoundly alter these structural and functional properties. In this study we have evaluated the response of cultured MEC to inflammatory mediators.

MEC were grown in a modified Iscove's medium to which histamine (10^{-5}M), IL-1 (5u/ml) or γINF (100u/ml) were added either singularly or in combination. Structural changes were assessed by light and electron microscopy, while functional alterations were determined by immunocytology.

When exposed to inflammatory mediators, MEC undergo mesenchymalization and deploy an elaborate glycosaminoglycan-rich reticular extracellular matrix (ECM). Factor VIII, absent in stimulated MEC, is found bound to the ECM. The morphologically altered MEC stain positively with anti-DR, anti-macrophage and anti-Factor XIIIa antibodies. Factor XIIIa is also bound to the ECM. Acquisition of properties characteristic of the Mononuclear Phagocytic (former Reticulo-Endothelial) System by MEC in vitro suggests that under inflammatory conditions in vivo, endothelial cells may have the capacity to participate to a greater extent in immune reactions than has heretofore been considered.

EFFECTS OF GRANULCYTE MACROPHAGE-COLONY STIMULATING FACTOR ON HUMAN LANGERHANS CELLS. Jack Longley, Steven Kolenik, Tie Gang Ding, and Richard Edelson, Dept of Dermatology, Yale Univ.School of Medicine, New Haven, Ct.

To determine how granulocyte macrophage-colony stimulating factor (GM-CSF) affects the expression of the Langerhans cell (LC) surface protein CD1a, epidermal cell (EC) suspensions were enriched for LC by density gradient centrifugation and cultured in the presence of 10 ng/ml GM-CSF. On isolation, 30% of cells typically expressed CD1a. By the fifth day of culture, CD1a expression had dropped to 4.2% of cells cultured in GM-CSF, while 9.5% of control cells were CD1a+. Viability was identical in cytokine treated and control cultures. GM-CSF consistently decreased CD1a expression by LC and by thymocytes, another CD1a+ cell type. Other experiments were performed to determine the effects of GM-CSF on LC function. LC enriched EC were cultured with GM-CSF for 72 hours, the cytokine containing medium was replaced with medium containing purified allogeneic T-cells and proliferation measured. GM-CSF treated EC induced 2 to 3 times more proliferation than EC cultured without additional cytokines. GM-CSF treated EC also stimulated purified CD8+ T-cells. T-cell proliferation was blocked by monoclonal antibodies reactive with either class I or class II major histocompatibility (MHC) proteins but not those reactive with CD1a. These studies demonstrate that GM-CSF, a cytokine known to be produced by keratinocytes *in vitro*, can affect the phenotype and function of human LC. Furthermore, the novel observations of decreased proliferation in the presence of anti-class I MHC antibodies and of the stimulation of CD8+ T-cells suggest that LC may be able to influence the immune system through other circuits besides the classically described class II MHC mediated, CD4+ T-cell pathway.

COMPARISON OF SERUM LEVELS OF 3α ANDROSTANEDIOL GLUCURONIDE IN CHINESE VS. CAUCASIAN SUBJECTS. D.P. Lookingbill, L.M. Demers, C. Wang, D.K. Murdock, A. Leung, and R.J. Santen, Pennsylvania State Univ., College of Medicine, Hershey, PA and Department of Medicine, Univ. of Hong Kong, Hong Kong.

Serum levels of 3α androstanediol glucuronide (3α diol G) may serve as a marker of peripheral tissue androgen action, wherein testosterone (T) is intracellularly converted by 5α reductase to dihydrotestosterone (DHT) which is ultimately metabolized to 3α-diol G and secreted from the cell. A reduction in levels of 5α-reductase might explain the low frequency of acne, scant amounts of facial and body hair, and decreased frequency of prostate cancer observed in the Chinese. Accordingly, we examined, in a pilot and an ongoing follow-up study, a sampling of Chinese (CH) and Caucasian (CA) male (M) and female (F) subjects. Each result is expressed in ng/dl and represents the mean + SEM.

	CH-M (n=38)	CA-M (n=38)	p-value	CH-F (n=27)	CA-F (n=26)	p-value
Total T	633±32	648±29	n.s.	48±3	59±7	n.s.
3αdiol G	471±29	1030±98	.0001	292±22	459±45	.001

In a subset examined clinically, we found higher mean chest hair scores (0-4 + scale) in 17 CA-M (2.8) than in 18 CH-M (0.3). Acne scores (scale 0-3+) were also higher in the CA-M (0.8) than in CH-M (0.4). The clinical scores were similar between groups in a total of 19 women. These results support the hypothesis that there is a difference in 5α-reductase activity between the two racial groups studied.

ANTIBODIES (Abs) TO ONCHOCERCA VOLVULUS CROSS-REACT WITH A HUMAN RO/SS-A (RO) AUTOANTIGEN (Aag). F.A. Lux, D.P. McCauliffe, D.W. Buttner*, J.D. Capra, R.D. Sontheimer, and T-S Lieu, Depts. of Derm. and Micro. Univ. of Texas Southwestern Med. Ctr., Dallas, TX, and Bernhard-Nocht-Institut fur Schiffs- und Tropenkrankheiten, Hamburg, FRG*

We have cloned and sequenced the gene for a human 46 kD Ro Ca^{++}-binding Aag which migrates aberrantly in SDS-PAGE at 60 kD. The sequence of this 46(60)kD Ro protein was found to have significant homology with λRal-1, a recombinant cDNA clone which corresponds to a major antigen (Ag) of the infective larvae of O. volvulus, the infectious agent of onchocerciasis (Onc). To study possible crossreactivity of Abs produced during the course of this disease to the human 46(60) kD Ro Ag, sera from 40 Liberian Onc patients who had no clinical evidence of autoimmune (Aim.) disease were tested in an ELISA assay. 26 of these sera had elevated IgG ELISA binding levels against the native human 46(60)kD Ro Ag as well as several Ro synthetic peptides. Onc sera also bound to the 46(60)kD Ro protein by immunoblotting, as well as to a COOH-terminal domain produced by V8 protease cleavage. By contrast, sera from patients with Aim. disorders bound mainly to the NH2-terminal domain. Anti-Ro sera characteristically coprecipitates the hY RNAs. Onc sera which had increased anti-Ro binding levels precipitated RNAs with the same electrophoretic pattern as the hY RNAs. In addition, the band corresponding to hY3 hybridized to an oligonucleotide probe representing a 24 base segment of hY3. These studies document that Onc patients produce Abs which cross-react with a 46(60)kD human Ro Aag and raise the possibility that infectious organisms such as O. volvulus may play a role in triggering or exacerbating a Ro-directed Aim. response.

UVA PROTECTION IN HUMAN EPIDERMIS: COMPARISON OF THREE SUNSCREEN FORMULATIONS. N.J. Lowe, M.M. Mobayen, T. Bourget, Southern California Dermatology and Psoriasis Treatment Center, Santa Monica, CA. S.H. Dromgoole, Herbert Laboratories, Irvine, CA.

Ten healthy adults participated in an investigator-masked study designed to determine the UVA protection of three sunscreen formulations by measuring UVA transmission through normal, suction blister-derived human epidermis. The formulations evaluated were: 1) Avobenzone + padimate O in a lotion formulation; 2) Octyl methoxycinnamate + octyl salicylate + oxybenzone + titanium dioxide in a cream formulation; 3) Octocrylene + octyl salicylate + oxybenzone in a cream formulation. Four suction blisters (each 7.5mm in diameter) were raised on the abdominal skin of each subject. The sunscreens were applied to the blister sites (2μl/cm^2) according to a randomization schedule; the fourth blister was not treated. After 15 minutes, the blister "roofs" were excised and placed on a UV-transparent plastic grid. The samples were then irradiated with UVA light from a filtered xenon arc solar simulator. The transmission of UVA light through the samples was measured with a spectroradiometer. The UVA protection of each formulation in each subject was expressed by a transmission protection factor, calculated by dividing the UVA transmission through the unprotected blister sample by the UVA transmission through the sunscreen-treated blister samples. Mean UVA transmission protection factors for the three formulations identified above were 6.2, 3.6, and 2.3, respectively. Thus, the formulation containing the UVA absorber avobenzone (absorption maximum at 358nm) provided 2 to 3 times as much UVA protection as the other two formulations.

COMPARISON OF GLYCOSAMINOGLYCANS (GAG) IN HUMAN PRE- AND POST-AURICULAR SKIN. N.B. Lyon, H. G. Garg*, E. Lippay*, R. W. Gange and I.E. Kochevar. Department of Dermatology, Harvard Medical School; and *Shriners Burns Institute. Boston, MA.

Chronic exposure of skin to sunlight induces elastosis and increased GAG. To evaluate the effect of chronic sun exposure on the amounts and distribution of GAG, total uronic acid (UA) was measured in the pre- and post-auricular skin of 5 patients undergoing face lift surgery. To determine whether GAG types were altered, individual specimens from 3 patients were evaluated using cellulose acetate electrophoresis. Whole skin was digested with papain prior to UA analysis. In all but one 46 year old patient, total UA was higher in pre-auricular, chronically sun-exposed skin compared with the protected post-auricular skin from the same patient. In each case, dermatan sulfate, heparin, heparan sulfate, chondroitin sulfate and hyaluronic acid were present in both sun-exposed and non sun-exposed skin, but the relative amounts of GAG types were altered in the sun-exposed skin. No trends were seen in the altered GAG distribution.

	Uronic acid (μg/gram (wet weight))		
Patient age (yrs)	Post-auricular	Pre-auricular	Ratio
46	102.0	153.0	1.5
46	141.5	128.1	0.9
58	156.5	193.5	1.2
60	282.6	573.7	2.0
69	82.8	150.3	1.8

DEVELOPMENT OF THERAPEUTIC IMPLANTS: PHARMACOKINETICS IN A MURINE TUMOR MODEL SYSTEM. . Edward Luck, Ning Yu, and Dennis Brown, Matrix Pharmaceutical, Inc., Menlo Park, California.

A mouse tumor model system was used in preclinical studies to evaluate the pharmacokinetics and efficacy of therapeutic agents administered by Therapeutic Implants (TI, Matrix Pharmaceutical, Inc.). TI are comprised of a carrier matrix, vasoactive modifier and either 5-fluorouracil (5-FU) or vinblastine (VLB). TI are under development as a local intralesional treatment for cutaneous neoplasms and various hyperproliferative diseases.

C3H female mice bearing transplantable RIF-1 fibrosarcomas (100-150 mm^3) were treated by intratumoral (i.t.) injection of 5-FU TI or VLB TI. Antitumor effect was evaluated for single doses and compared with intraperitoneal (i.p.) free drug administration by a tumor regrowth assay. Blood and tumor samples were collected at designated intervals after injection. Radiolabelled drugs were quantitated by liquid scintillation counting.

TI administration achieves high tumor concentrations which are 4.35 fold greater for 5-FU and 3.1 for VLB (area under curve) than free i.t. drug over a 24 hour period. Since TI provide sustained tumor drug levels, blood drug levels were less (79% for 5-FU and 51% for VLB) than that of free i.t. drug. VLB TI treatments produced a 180-fold greater tumor drug concentration than systemic administration of free drug.

These mouse studies indicate that TI offer advantages over free drug administration by providing sustained drug release at the treatment site and limiting systemic exposure to drug exposure. Furthermore, TI have been shown to be feasible for targeted administration of therapeutic agents in the clinical setting.

POROKERATOSIS: ABERRANT PROLIFERATION OF LUMEN-FORMING CELLS IN SQUAMOUS EPITHELIUM OF SKIN. Alice P. Ma, Andrew Tranvan and Annette M. Dineen. Section of Dermatology, Department of Medicine, The University of Chicago, Chicago, Illinois.

The present investigation was based on the postulate that porokeratosis lesions represent clonal growth of epidermal cells, as proposed by Reed and Leone in 1970. Comparative studies were carried out using immunocytochemical staining, epidermal cell culture and non-equilibrium pH gel (NEpHG) electrophoresis analysis of the keratins extracted from five patients with various types of porokeratosis. Positive type IV collagen staining on the stratum corneum was found in lesional skin specimens of all patients but not in normal controls. A search for connections between type IV collagen in the basement membrane of epidermis and the skin surface disclosed infrequent intraepidermal streaking of type IV collagen and one positively stained transepidermal acrosyringium. Positive intraepidermal laminin staining in porokeratosis lesions confirmed the transepidermal passage of basement membrane materials. Epidermal cells cultured from lesional skin showed low plating efficiency and all colonies exhibited intracytoplasmic vacuole formation, and excessive top cell shedding. NEpHG electrophoresis of keratins extracted showed that lesional profiles not only contained keratins normally present in glabrous skin but also K9, K19 and some additional proteins. K9 had been immunolocalized to periductal cells in previous studies. Our findings, taken together, strongly suggest that porokeratosis epidermis consists of epithelial cells with lumen forming ability.

DIFFERENTIAL EXTRACELLULAR EXPRESSIONS OF CALPACTIN I HEAVY CHAIN IN SKIN EPIDERMAL CELLS AND FIBROBLASTS. Alice S-P Ma, Section of Dermatology, The University of Chicago, Chicago, Illinois.

Monoclonal antibody CP-I, raised against human skin epidermal cells, immunoprecipitated a 36kD protein which was proven to be Calpactin I heavy chain. The protein, purified using antibody affinity column, when cleaved with cyanogen bromide and sequenced, yielded 33 amino acid sequence identical to sequence 171 to 203 of Calpactin I.

Calpactin I, also known as Lipocortin II, has been studied by various groups as a major substrate of src tyrosine-protein-kinase and an inhibitor of human phospholipase A_2. Although its cDNA sequence data and many of its biochemical properties were known, its precise functions remain unclear. We confirmed previous reports of its wide tissue distribution and its cytosolic cellular localization using CP-I. However, not reported before was our finding that Calpactin I was expressed intercellularly in epithelial cells and at some cell contact points in fibroblasts using pre-fixation method.

To further test whether Calpactin I resides at cell contact points, both cultured keratinocytes and fibroblasts were perturbed using 0.02% EDTA into cell suspensions and membrane cytosolic extract were compared to those from cultures removed by rubber policemen. There was a distinct quantitative decrease of calpactin I in keratinocyte pellets, but not in the two fibroblast extracts. These findings suggest a functional role of Calpactin I in spatial arrangement of cells during tissue organization and the possibility of Calpactin I's role as a phospholipase A_2 inhibitor.

HIV TRANSCRIPTS DETECTED IN HIV-ASSOCIATED KAPOSI'S SARCOMA AND PSORIASIS. Stephen E. Mahoney, Madeleine Duvic, Brian J. Nickoloff, and Dorothy E. Lewis, Depts of Dermatology, Medicine, Pathology, Microbiology and Immunology, Univ. of Texas at Houston, Univ. of Michigan, and Baylor College of Medicine, Houston, Texas.

Kaposi's sarcoma (KS) and psoriasis are cutaneous manifestations of HIV infection of unknown pathogenesis which contain numerous dermal dendrocytes expressing Factor XIIIa. Transgenic mice containing HIV TAT or proviral sequences develop KS and psoriasiform epidermal hyperplasia resembling these disorders. To examine the hypothesis that HIV may have a direct role in the pathogenesis of HIV associated KS and psoriasis, we combined in situ hybridization with a sensitive laser microscopy scanning system to detect HIV RNA transcripts in lesional skin. Skin biopsies from 14 HIV infected and 17 HIV negative patients were hybridized to a 35S-labeled antisense HIV riboprobe, as well as negative (pGEM) and positive (keratin) control probes. After analysis for in situ hybridization, biopsies were stained for factor XIIIa. Five of 13 HIV infected psoriasis biopsies and 2 of 3 infected KS biopsies had HIV transcripts within the epidermis or dermis compared to 0 of 17 HIV uninfected biopsies including 4 with psoriasis and 1 with KS ($p=.009$). Colocalization of HIV probe within Factor XIIIa positive dendritic cells was observed in both KS and psoriasis lesions. The presence of HIV transcripts within dermal dendrocytes of psoriasis and KS lesions suggests a direct role for both HIV and dermal dendrocytes in the pathogenesis of these disorders.

INHIBITION OF PROTEIN KINASE C ACTIVITY BY PHOTOACTIVATED PSORALENS. Wei Ma and Jeffrey D. Laskin, Department of Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey

Psoralens are photosensitizing chemicals that are active against a wide variety of epidermal proilferative disorders. We have found that these compounds are active at the level of the cell membrane and are potent inhibitors of epidermal growth factor (EGF) receptor binding and EGF receptor tyrosine kinase activity in epidermoid cells. This effect was temperature-dependent and required intact cell membranes. Inhibition of EGF binding and receptor kinase activity could not be prevented by the protein kinase C inhibitors sphingosine or H7 (1-(5-isoquinolinylsulfonyl)-2-methyl-piperazine) or in cells in which protein kinase C was down-regulated by phorbol ester tumor promoter pretreatment. To determine if the psoralens had direct effects on protein kinase C activity, we prepared membranes from HeLa cells treated with 4,5',8-trimethylpsoralen and ultraviolet light (PUVA) and assayed for the enzyme by immunoblotting and in vitro kinase methods. We found that PUVA caused an inhibition of protein kinase C activity without altering the amount of enzyme in membranes. Neither the psoralen nor UV light alone were effective in inhibiting protein kinase C activity. These data suggest that changes in protein kinase C activity may play a role mediating the biological effects of PUVA at the cell surface membrane.

MATURATION PATHWAYS OF FILAGGRIN IN NORMAL AND ABNORMAL EPIDERMIS. Motomu Manabe, Beverly Dale, John P. Sundberg, and Tung-Tien Sun, Epithelial Biology Unit, Depts. of Dermatol. and Pharmacol., New York University School of Medicine, New York, NY; Depts. of Periodontics and Oral Biol., University of Washington, Seattle, WA; The Jackson Laboratory, Bar Harbor, ME.

Keratohyalin granules (KH) are important morphological markers of keratinized epithelium. Using antibodies highly specific for the filaggrin subunit of these granules, we performed EM localization studies to define the ultrastructural involvement of filaggrin in KH formation. In normal mouse epidermis, filaggrin is first associated with KH in granular cells, and later with larger KH clusters in transitional cells. In lower cornified cells, filaggrin disperses and becomes evenly distributed in the entire cytoplasm. Finally in upper cornified cells filaggrin becomes undetectable. The disappearance of filaggrin in upper cornified cells correlates well with the dissociation of keratin filament bundles, thus providing strong evidence for the first time that filaggrin may be involved in keratin filament aggregation in vivo. In another series of experiments, we examined the alteration in filaggrin maturation in a recently described mutant mouse ("flaky skin') whose epidermis undergoes abnormal keratinization. The "dispersion" step of filaggrin in this mutant epidermis appears to be defective, resulting in the accumulation of a large number of superficial cell layers with a filaggrin pattern characteristic of transitional cells. This mutant mouse serves as an excellent, new model for studying filaggrin maturation in transitional cells.

CHARACTERIZATION OF THE CERAMIDES OF MOUSE EPIDERMIS AND ORGANOTYPIC MURINE KERATINOCYTE CULTURES. Kathi C. Madison, Donald C. Swartzendruber, Philip W. Wertz, and Donald T. Downing, Dep't. of Dermatology, Univ. of Iowa College of Medicine, Iowa City, Iowa.

Ceramides (CER) are the dominant component of the stratum corneum intercellular lipid lamellae which are responsible for the epidermal permeability barrier. Only pig and human epidermal CER have been characterized and the structures of the CER of cultured keratinocytes have not been previously investigated. In this study we have examined the CER of intact mouse epidermis and compared them to the CER synthesized by organotypic mouse keratinocyte cultures (MKC).

Lipids were extracted from mature MKC [JID 90:110-116, 1988] and isolated mouse epidermis; individual CER were isolated and various analytical methods were used to determine their chemical structures.

Both mouse epidermis and MKC contained 5 CER, CER 1 being the least polar and CER 5 the most polar. CER 1 was a very-long-chain ω-OH CER with an ester-linked non-OH FA. CER 2 contained medium-length saturated non-hydroxy FA and CER 5 contained short chain α-OH FA. The structures of CER 1, 2, and 5 were analagous to those of pig and human epidermis. Mouse CER 3 was quite unusual, containing β-OH FA, a structure not previously identified among mammalian CER. Culture CER 3 was composed of ω-OH FA with a chain length distribution similar to that of CER 1. Mouse CER 4 was composed of FA with TLC mobility like OH-FA but with different chemical reactivity and remains partially characterized. Culture CER 4 was present in quantities too small for analysis. All CER in mouse epidermis and MKC contained only sphingosine bases whereas pig and human CER also contain phytosphingosine. The results indicate that considerable diversity of CER structures occurs among mammalian species and that cultured keratinocytes may only partially reproduce the in vivo complement of CER.

USE OF A POLYMORPHIC DNA PROBE FOR DIAGNOSIS OF DYSKERATOSIS CONGENITA. WR Mann[1], VS Venkatraj[1], DM Carter[1], AD Auerbach[1], [1]The Rockefeller University, New York, NY.

Dyskeratosis congenita (DC) is a rare genodermatosis whose primary defect remains unknown. Characterized by reticulated skin hyperpigmentation, nail dystrophy, mucosal leukoplakia, pancytopenia and cancer proneness, it exists in both X-linked recessive and autosomal dominant forms. The aim of this study was to determine whether a polymorphic DNA probe, DXS52 (St14-1), known to be tightly linked to the X-linked DC gene could be used to assist the genetic counselling of a 29 year old woman who had had a spontaneous abortion, and whose two brothers (previously reported in the literature) had died of the X-linked recessive form of DC. Her parents were also deceased. A fibroblast cell line from one brother which was frozen for more than a decade was successfully reactivated. DNA was obtained from this cell line and from the woman, her husband, her male abortus, two of her maternal uncles, and two of her maternal aunts. The DNA samples were digested with Taq I and subjected to electrophoresis, Southern blotting and hybridization to St14-1 according to standard techniques. The autoradiograph showed that the woman shared an allelic band with her brother, aunts and uncles, which she did not pass on to her abortus. Assuming that no recombination had occurred between the disease locus and the locus defined by St14-1 we conclude that the abortus was unaffected by DC and that prenatal diagnosis using this and other closely linked probes will be of use in the woman's genetic counselling.

CORRELATION OF PLASMA MEMBRANE VISCOSITY WITH KERATINOCYTE FATTY ACID COMPOSITION. Cynthia L. Marcelo, Elizabeth A. Duell, Susan B. Klein, Lenore M. Rhodes, William R. Dunham and Richard H. Sands, Dept. of Dermatology and Biophysics Research Division, Univ Mich, Ann Arbor, MI.

Growth of adult human keratinocytes in serum free medium yields cells almost totally devoid of essential polyunsaturated fatty acids (FA). In this state the basaloid monolayers grow very rapidly, not forming the slower growing stratifying, differentiating cultures seen using serum-containing (+FA) medium. Since shifts in membrane viscosity should result from the changes in membrane FA composition, the cell growth and membrane viscosity of keratinocytes grown in control and 6 fatty acid containing media were investigated. Cells were grown in MCDB 153 containing no FA (0X), 1 to 100 μM 18:2 or 20:4 (+20:4 or +18:2), 5 μM 18:2 and 16:0 μM (1X), 10 μM 18:2 and 5 μM 16:0 (2X/1X), 10 μM 18:2, 5 μM 16:0 and 20:4 (2X/1X/1X) and 15 μM 18:2 and 5 μM 16:0 (3X/1X). Lipids were extracted into methanol-chloroform, methylated and analyzed by HPLC; TLC separated polar lipids (membrane FAs) were also analyzed. Growth in +18:2 or +20:4 media greatly inhibited cell growth. Cells grown in 1X, 2X and 2X/1X media grew 2-fold the 0X medium rate. 3X/1X medium cultures grew equal to the 0X controls. HPLC analysis showed significant increases in all the FA's in the 1X to 3X/1X medium grown cells. Significant increases in some polar lipid unsaturated FA's were seen. In this extreme EFAD state, cell viscosity correlates inversely with the 14:0, and directly with the 16:0 and 18:0 content. The data support that in this EFAD in vitro state, the keratinocyte adapts in part by utilizing 14:0 to maintain cell membrane viscosity levels in a state allowing expansive cell growth.

CHARACTERIZATION OF HUMAN KERATINOCYTE PROTEIN KINASE C. Mary Steidl Matsui and Vincent A. DeLeo, Department of Dermatology, Columbia University, New York, New York.

Human keratinocyte (HK) growth and differentiation are regulated by extracellular Ca^{+2} concentration. Protein kinase C (PKC), a Ca^{+2} activated, phospholipid dependent protein kinase, has been identified as a key regulatory enzyme in mediating growth and differentiation, and is the major cellular receptor for 12-O-tetradecanoylphorbol-13-acetate (TPA). TPA may have an indirect rather than a direct effect on HK in vivo, and stimulates HK differentiation rather than growth. The present series of experiments demonstrates that in the absence of other growth factors, 1.6mM external Ca^{+2} (switched from .15mM Ca^{+2}) stimulated a decrease in phorbol binding at 30min which lasted for at least 3h, but stimulated a 1.5-2 fold increase by 24h. Pretreatment with 200ng/ml TPA for 30min decreased phorbol binding in cells exposed to either high or low Ca^{+2} for 30min or 24h, but did not downregulate epidermal growth factor binding. Immunoblotting techniques were used to determine PKC distribution and abundance and revealed a protein of about 110 kDa that coeluted with the expected 80 kDa PKC from DEAE sephacel, phenylsepharose, and hydroxyapatite columns. This protein may play an important role in the unique responses of HK PKC, resulting in significant differences between this cell type and the classic mouse skin intiation/promotion model.

CLINICAL AND PHOTOBIOLOGICAL EVOLUTION OF PERSISTANT PHOTOSENSITIZATION WITH CORTICOSTEROID-PUVA THERAPY OR AZATHIOPRINE THERAPY : 3 CASE REPORTS. Marguery MC., Sottil JP., Bazex J., Department of Dermatology, La Grave Hospital, Toulouse, France.

Current therapy for persistant photosensitization (PP) is the association corticosteroid-PUVA therapy (CP) (1,3,4) or azathioprine(A) (5,6). We studied short and long term clinical and photobiological parameters (MED/UVB ; UVA phototest) in 3 patients with PP, 2 of whom had CP therapy and the other had A. Our goal was to clarify effectiveness, advantages and disadvantages of the 2 modes of therapy.

Upon analysis of our data we obtained the following results : 1) A and CP had comparable effectiveness. 2) advantages of A therapy were numerous including ease of prescription and quality of life in that the therapy is ambulatory. A is also useful when systemic corticosteroids are contraindicated. 3) disadvantages of A are the risk of hepatic and bone marrow toxicity.

In conclusion, the advantages of A therapy outweigh the disadvantages and with no sacrifice of effectiveness. Consequently, we propose a new protocol : induction of remission with A then maintenance PUVAtherapy. We also feel that codification of term therapeutic regimens for PP requires an extended study of photobiological parameters.

DETECTION AND PURIFICATION OF A HIGH-MOLECULAR-WEIGHT PROTEINASE WITH MULTICATALYTIC PROPERTY FROM RAT EPIDERMIS. T. Matsuyama, M. Shimozuma and K. Fukuyama, Department of Dermatology, University of California, San Francisco, CA.

A high-molecular-weight proteinase with multicatalytic property has been characterized from many cell sources. We report the isolation of proteins from rat epidermis which cleave substrates for more than one type of endopeptidase. Two fractions, spinous and granular cells (SGC), and cornified cells (CC) were obtained from Sprague-Dawley strain rat epidermis. Proteins extracted in 25 mM Tris-HCl, pH 7.5, containing 1 mM 2-mercaptoethanol and 20% glycerol were fractionated on a Bio-Gel A 1.5m column. Hydrolase activity on Suc-Leu-Leu-Val-Tyr-AMC was detected in proteins with Mr=600 kD from SGC and Mr=400 kD from CC. The active peak from both cell sources eluted at 0.4 M NaCl from a Mono Q column using FPLC was separately purified to a single peak on a HPLC G3000SW column. The enzyme was purified 234 fold from SGC and 546 fold from CC. The enzyme hydrolyzed Z-Ala-Arg-Arg-AMC and MeOSuc-Phe-Leu-Phe-AMC in addition to Suc-Leu-Leu-Val-Tyr-AMC. Km values of the SGC enzyme for the substrates were 0.13, 0.047 and 0.18 mM, respectively. The activity toward Suc-Leu-Leu-Val-Tyr-AMC and MeOSuc-Phe-Leu-Phe-AMC was totally abolished with leupeptin. On the other hand, leupeptin effectively blocked hydrolysis of Z-Ala-Arg-Arg-AMC, but not the activity on the other two substrates. These findings indicate that the molecular weight of an endopeptidase with several catalytic properties in SGC is reduced in CC during keratinization without changing its enzymic activity.

EVALUATION OF A COLLAGEN MATRIX SPONGE IN FULL THICKNESS WOUNDS IN THE PRESENCE OF TWO WOUND PATHOGENS. D.A. Marshall, J. Patti, A.L. Cazzaniga, P.M. Mertz, Univ. of Miami Sch. of Med., Dept. of Dermatology, Miami, FL. G. Ksander, Collagen Corp. Palo Alto, CA

New tissue matrix materials are being developed for use in wound healing and tissue augmentation. There is concern that such materials might promote infection. The proliferation of two bacterial pathogens in biopsy wounds implanted with a collagen sponge and covered with Telfa dressing or wounds covered with Telfa dressing alone was examined. Twenty-four punch biopsy wounds (20mm dia.) were made on the backs of each of 4 domestic pigs. Wounds were inoculated with 10^6 colony forming units (CFU) of either *Staphylococcus aureus* or *Bacteroides fragilis*. Only one pathogen was used per animal. Collagen matrix sponge and/or dressing were applied immediately and on Days 3, 7, 10, 14, and 17. Wounds were also examined for clinical signs of infection (erythema, pus, etc.) and 8mm diameter biopsies were removed for pathogen quantitation at these times. Total average numbers of pathogens for days evaluated (Days 3, 7, 10, 14, and 17) are shown below:

	Mean logarithm CFU/Gram Tissue ± S.D.	
Pathogens	Collagen and Telfa	Telfa Only
S. aureus	6.8 ± 0.8	7.4 ± 0.6
B. fragilis	7.3 ± 0.6	6.8 ± 1.0

No wounds had clinical signs of infection. Pathogen multiplication from both treatment groups was similar. The results suggest that collagen sponge implantation in full thickness wounds does not encourage the growth of *S. aureus* and *B. fragilis* compared to wounds treated with conventional Telfa dressing alone.

BULLOUS PEMPHIGOID: EOSINOPHIL, NEUTROPHIL AND MAST CELL DEGRANULATION IN LESIONAL TISSUE. B Maynard,MS Peters,JH Butterfield and KM Leiferman,Dept of Dermatology and Medicine, Mayo Clinic, Rochester MN

Histopathologic changes in bullous pemphigoid include alterations in mast cells, neutrophil and eosinophil infiltration, and finally blister formation. Dermoepidermal separation likely occurs either as a result of direct cytotoxic action or because of the effect of lysosomal proteolytic enzymes. To determine whether mast cell, neutrophil and eosinophil degranulation occur in bullous pemphigoid, specific granule proteins were localized by indirect immunofluorescence using antibodies to mast cell tryptase, neutrophil elastase, eosinophil granule major basic protein (MBP), eosinophil-derived neurotoxin (EDN) and eosinophil-cationic protein (ECP) in eight specimens of lesional skin. Although mast cells were detected, few degranulated mast cells were observed. Neutrophil infiltration was observed in most of the biopsy specimens but there was minimal or no extracellular neutrophil elastase except one specimen which showed prominent neutrophil infiltration and extracellular elastase deposition. Eosinophils were observed in all of the biopsy specimens, and there was extracellular deposition of MBP, EDN and ECP in the dermis in 7 of the 8 specimens, especially prominent at the edges and beneath blisters. MBP was measured by radioimmunoassay in the serum and bullous fluid from one patient and the levels were 198 ng/ml (normal serum range <600 ng/ml) and 2970 ng/ml respectively. The results show that mast cells are not prominent in lesional skin of bullous pemphigoid. Although neutrophil infiltration occurs along with eosinophil infiltration, the neutrophil does not appear to be the major effector cell in association with tissue damage. Rather, the results suggest that eosinophil degranulation contributes to tissue damage in bullous pemphigoid.

THERMOTOLERANT HUMAN KERATINOCYTES IN VITRO ARE RESISTANT TO ACUTE ULTRAVIOLET LIGHT (UVB) EXPOSURE. Edward V. Maytin and Joanne M. Wimberly, Wellman Laboratories, Dept. of Dermatology, Harvard Med. School, Massachusetts General Hospital, Boston, MA

We have recently shown that the heat shock response in normal, cultured human keratinocytes (induced by a mild thermal conditioning of 42° for 1 hr) makes the cells resistant, or "thermotolerant," to a later high-temperature challenge (e.g. 49° for 20 min). To investigate the specificity of this protective reponse, we have begun to examine agents of injury other than heat. Here the effect of the heat shock response upon survival after UVB injury was examined. Confluent keratinocytes in quadruplicate 0.3 cm^2 wells, in KGM medium (Clonetics), were heated at 42° for 1 hr, returned to 37°, then irradiated at 6 hr (a time when thermotolerance is maximal) in clear PBS buffer, with UVB (290 to 310 nm) delivered by a modified Oriel 1000-watt Xenon arc. At 24 hr, the viability of cells measured by uptake of fluorescein diacetate decreased steadily with doses up to 200 mJ/cm^2. After UVB doses of 20, 60, and 100 mJ/cm^2, the thermotolerant cells displayed significantly greater viability (up to 2-fold) compared to their non-thermotolerant counterparts. The protective effect could not be ascribed to attenuation of UVB, since no morphological rounding in thermotolerant cells was seen. The protective effect was abolished by the presence of cordycepin, an inhibitor of mRNA synthesis, during the 42° conditioning. We conclude that heat shock can confer protection against UVB-induced lethality. The effect requires the synthesis of new mRNA's, perhaps encoding heat-inducible heat shock proteins.

PUVA MODULATES HUMAN CUTANEOUS MAST CELL RESPONSIVENESS. Ann McGowan-Tuskes, An Yen, Kim E. Barrett and Irma Gigli, Department of Medicine, Univ. of California School of Medicine, San Diego, California.

We have previously reported that photoactive substances plus UVA light have dual effects on rodent mast cells in vitro. High concentrations have a direct histamine releasing action while low concentrations inhibit histamine release in response to secretagogues without a cytotoxic effect. To investigate whether this mast cell-stabilizing activity also occurs in vivo, prick puncture skin test responses to codeine (3 - 60 mg/ml) a mast cell secretagogue, and histamine (1 mg/ml and saline as controls, were assessed in normal individuals before the ingestion of 10, 20 or 30 mg 8-methoxypsoralen and again 90 mins after psoralen administration in UVA-irradiated (PUVA) and non-irradiated skin. PUVA caused a decrease in wheal and flare responses to codeine relative to pre-psoralen responses, but did not alter the response to histamine. In subjects receiving 10 mg of psoralen (N=8), pre and post PUVA flare areas (means ± SEM) in response to 60 mg/ml codeine were 5.9 ± o.5 cm^2 and 3.0 ± 0.5 cm^2 respectively, a reduction in flare of 49 ± 7.1% (p 0.001). Inhibition was noted with all the codeine concentration used. Increasing the dose of psoralen did not enhance inhibition. This effect of psoralen plus UVA is most likely mediated at the level of the mast cell rather than the vasculature, since responses to histamine in same subjects were not reduced by PUVA (flare pre and post PUVA 3.8 ± 0.7 and 4.1 ± 0.4 cm^2 respectively). Our findings may partly explain the efficacy of PUVA in mast cell-mediated skin disorders.

GROWTH CHARACTERISTICS AND ANTIBIOTIC RESISTANCE OF MYCOBACTERIUM HAEMOPHILUM. M. E. McBride, A. H. Rudolph, B. Brown, R. J. Wallace, Department of Dermatology, Baylor College of Medicine, Houston, Texas, Department of Microbiology, University of Texas Health Science Center at Tyler, Tyler, Texas.

Mycobacterium haemophilum, a recently described atypical species of Mycobacterium, was isolated from a skin biopsy of a patient recovering from a coronary bypass and was unresponsive to treatment with anti-mycobacterial drugs. This organism is characterized by the requirement for hemin and hence will not grow on routine media for Mycobacteria. Furthermore, antibiotic susceptibility testing cannot be done in the routine manner for the same reason. The purpose of this study was to evaluate various media, culture conditions, and different sources of hemin to achieve optimum growth and devise a method for antibiotic susceptibility testing. Three basal media: Casman's, GC, and Charcoal were compared with Middlebrook Seven H 11. Hemin sources were sheep's blood, whole and lysed, haemoglobin and hemin. Although the organism required 6 weeks incubation for primary isolation on sheep's blood agar, growth was achieved in 7 days on Casman's agar base with lysed sheep's blood. Using a medium supplemented with hemin, the isolate was found to be resistant to augmentin, erythromycin, ethambutol, moderately resistant to doxycycline, minocycline and amykacin and sensitive to ciprofloxacin and trimethoprin sulfate, a sensitivity pattern unusual for Mycobacterium spp. The patient has responded to treatment with ciprofloxacin.

RECONSTITUTION OF IRRADIATED MICE WITH GAMMA/DELTA T CELLS ISOLATED FROM THE FOETAL THYMUS GLAND. Hilary McKenna, James Watson, and Nicholas Birchall, Dept. of Immunobiol., Auckland Univ., NZ.

Various strains of mice were time-mated and sacrificed at 12-14 days. Foetal thymus glands were dissected and 1/2 lobes placed in submersion culture in round-bottomed wells. Culture medium was 10% FBS in complete RPMI supplemented with 20 ng/ml rhIL-7. IL-7 is a recently cloned B and T cell growth factor. After 8 days culture, a single-cell suspension of FT was prepared. These cells were analyzed by FACS. The cells were thy1, B220, NK1.1, Pgp-1 and J11D positive double negative (CD4 and CD8) $\gamma\delta$ T cells (Northern blot and FACS staining with F536 and Ly6c). This FACS profile suggests a developmentally early cell. Thus, we asked whether IL7-treated $\gamma\delta$ T cells were capable of reconstituting a sub-lethally irradiated mouse, i.e., are these cells pleuripotent? Congenic C57BL10/Kathy and KA mice were used. The two strains differ at the thy1 locus only (thy1.2 and thy1.1); thus, donor cells can be differentiated from those of the host. Thy1.2 cells were injected into thy1.1 split-dose irradiated recipients intrathymically or intravenously. Varying concentrations of cells were injected (10^3-10^6 cells). FACS analysis of thymus, lymph nodes, spleen and skin was performed at 3-day intervals after injection using the congenic thy markers. Cells of donor origin were found in the skin in 50% of animals from day 8 and persisted long-term. Numerical calculations suggest this population of cells expanded after injection. No cells of donor origin were found in the thymus. Colonies of host cells were macroscopically evident on the spleen after 8-10 days. To assess cell migration, ^{51}chromium-labelled T cells were injected i/v or i/t and organ gamma counts performed after sacrificing the animals at various time intervals post-injection. Freshly isolated FT $\gamma\delta$ T cells migrated rapidly (15-20 min) to the skin, gut and lungs. A population of $V\gamma_3$ FT, however, homed preferentially to the skin. These findings suggest that the $\gamma\delta$ T cells are developmentally mature cells that migrate out and home directly to peripheral tissues. In summary, although the FT $\gamma\delta$ cells have a deve opmentally early phenotype, they do not appear to be pleuripotent and are incapable of reconstituting irradiated hosts. The cells appear to be developmentally mature cells that migrate to the skin as mature thy1+ DEC cells.

A HUMAN Ro/SS-A (Ro) AUTOANTIGEN IS THE HUMAN HOMOLOGUE OF MURINE CALRETICULIN, ONCHOCERCAL RAL-1 ANTIGEN, AND APLYSIA PROTEIN 407. D.P. McCauliffe, T-S Lieu, T. Kennedy, R.D. Sontheimer and J.D. Capra, Depts of Dermatology and Microbiology, UT Southwestern Med. Ctr., Dallas, Tx, and Center for Neurobiology and Behavior, Columbia University and Howard Hughes Medical Institute, New York, NY.

We have isolated a human cDNA clone which encodes a 46 kD Ro autoantigen that migrates aberrantly at 52-60 kD in SDS-PAGE. Synthetic oligonucleotides corresponding to this human Ro sequence were used to amplify the homologous gene from a murine B-cell cDNA library utilizing the polymerase chain reaction. The mouse cDNA encoded amino acid sequence was found to be 94% homologous to the human Ro sequence and is 100% homologous to murine calreticulin, a calcium binding protein which resides in the endoplasmic reticulum. Human Ro antisera were originally reported to produce cytoplasmic immunofluorescence, a pattern similar to that seen with calreticulin antisera and with a rabbit antiserum directed against the NH_2 end of our Ro protein. The RAL-1 antigen of Onchocerca volvulus, a human filarial parasite, was also found to be 63% homologous to human Ro. In addition, the human Ro protein has a similar molecular weight, isoelectric point and significant amino acid sequence homology to the Aplysia californica snail neuronal protein 407. These homologies suggest that this human Ro protein is highly conserved across species and has a very basic cellular function(s) which in part may involve calcium binding. The high degree of homology with a conserved parasitic antigen suggests that a foreign Ro protein homologue could possibly trigger an autoimmune response in humans.

NORMAL HUMAN SERUM AND FETAL BOVINE SERUM CONTAIN AN IL-1 INHIBITOR. R.C. McKenzie, C.B. Harley, S. Matic and D.N. Sauder, Depts. of Medicine and Biochemistry, McMaster University, Hamilton, Ont., CANADA

Natural inhibitors of IL-1 have been characterized from a variety of body fluids although their role in immunoregulation remains to be elucidated. The keratinocyte cell line COLO-16 constitutively produces factors with IL-1 activity, including IL-1α and IL-1β. IL-1 activity was 60% less if cells were maintained in media containing 10% fetal bovine serum (FBS) compared to media without serum 24h prior to harvest. Measurable IL-1 activity was inversely proportional to the FBS content of the growth media. The increased IL-1 activity in supernatants from cells maintained in serum-free media was not due to increased cellular levels of IL-1α or IL-1β mRNA. Serial dilutions of recombinant IL-1α and IL-1β were made in media containing 10% FBS or 10% normal human serum (NHS) and assayed by thymocyte proliferation. A 30-50% decrease in activity was noted for samples containing FBS or NHS, relative to controls. A partial purification of these inhibitors was made by gel filtration chromatography of FBS and NHS over a sephacryl 200 column. The inhibitory species from both FBS and NHS was found to have a molecular weight of ≈85kDa. These IL-1 inhibitors present in serum may not only interfere with assays for IL-1 but may also play a major regulatory role in IL-1 modulation.

INTERLEUKIN 8 IMMUNREACTIVITY IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMA. J. McLean[1], C. Feliciani[1], D. Rosenthal[1], E. Christophers[2], M. Sticherling[2], J.-M. Schröder[2] and D.N. Sauder[1]. Dept. of Medicine, McMaster University, Hamilton, Ont. CANADA and Dept. of Dermatology, University of Kiel, Kiel, FRG.

Cutaneous T cell Lymphoma (CTCL) is characterized histologically by a monoclonal proliferation of neoplastic T cells which localize in the skin. The mechanism for this T cell localization is unclear, however, recent studies have suggested that epidermal cytokines may influence T lymphocyte migration. Previous studies have shown interleukin 1 (IL-1) is T cell chemoattractant. Furthermore IL-1 is increased in skin of patients with CTCL as demonstrated by direct immunofluorescence. Recently a new cytokine, interleukin 8 (IL-8) has been shown to be an even more potent chemoattractant for T cells. IL-8 is present in human keratinocytes and can be induced by IL-1. To gain insight into the role of IL-8 in CTCL we performed direct immunofluorescence using a monoclonal antibody against recombinant IL-8. Five lesional biopsies from patients with clinical and histologically proven CTCL were studied. All studies consistently revealed intense intercellular staining with the antibody as compared to normal skin. In contrast to increased IL-8 immunoreactivity, IL-8 mRNA levels were not elevated in lesional skin from patients with CTCL. Thus IL-8 mRNA may be transiently expressed in lesional skin from CTCL but IL-8 protein may accumulate. These studies suggest that elevated cytokines such as IL-8 may play a role in the pathogenesis of CTCL.

ACTIVITY OF SANDIMMUNE (CYCLOSPORINE A) IN DERMATITIS-MODELS. Josef G. Meingassner, Assad Bavandi, and Gabor Petranyi, Department of Dermatology, Sandoz Forschungsinstitut, Vienna, Austria.

Sandimmune (Sandoz) was evaluated after topical application at concentrations of 0.01 - 10.0% in models (mice, guinea pigs or farm pigs) of UV-erythema, irritant dermatitis (caused by arachidonic acid, calcium ionophore A 23187, TPA and cantharidin), oxazolone- or DNFB-induced allergic contact dermatitis and mycotic skin infections. Also, activity against a subacute eczema in hairless rats, caused by dietary conditions, was assessed after peroral treatment. Drug efficacy was based on clinical observation, measurement of edematisation, skin color, temperature or microvascular perfusion (Laser Doppler flowmeter).

Sandimmune was shown to exhibit dose-dependent antiinflammatory effects in these dermatitis models (except the pig) when applied epicutaneously. The lack of topical activity in farm pigs was probably due to insufficient percutaneous absorption. Systemic activity also was shown in rats sufferring from dietetic-dependent eczema. These data demonstrate activity of Sandimmune in skin models other than the classical DTH reaction, suggesting interference with non-immunological processes.

A MODEL SYSTEM FOR THE DIFFERENTIATION OF SEBACEOUS CELLS. M.I. Mednieks, S.J. Laurent, S. Liao, A.R. Hand and R.L. Rosenfield, Department of Pediatrics; Ben May Institute and the Department of Biochemistry and Molecular Biology, Univ. of Chicago, Chicago, IL; Department of Pediatric Dentistry, Univ. of Connecticut, Farmington, CT.

Preputial glands from rats were used to develop an *in vitro* system for studying mechanisms of hormonal regulation of sebaceous cell differentiation (Rosenfield, J.Invest. Dermat.92, 1989; Mednieks et al. Adv. Cyclic Nuc. Prot. Phosph. Res., *21*, 1990, in press). Cells from dissociated tissue in monolayer and in colony-forming stages respond to stimulation by agents whose action is mediated via cyclic AMP. Generally, an increase (3 to 5 fold) in photoaffinity labeling of the regulatory (R) subunits of cyclic AMP-dependent protein kinase with 8-azido cyclic AMP was seen when compared to unstimulated controls. Specifically, stimulation with isoproterenol (IP) or forskolin resulted in 50 to 300% greater activity of RII. An isoform (RII_{54}) appeared after stimulation with IP which was absent in unstimulated preputial cells or in cells from the epidermis. Additionally immunological localization studies of epithelial cells from the preputial gland in culture showed distribution of marker keratin (K4) which is unique for sebaceous cells. Electron microscopic immunogold labeling using monoclonal antibodies to fusion proteins encoding specific domains of the rat androgen receptor (AR MAb) showed reactivity mainly in the nuclei of acinar cells of the preputial gland. Western blots of nuclear extracts from preputial and prostate cells in culture showed reactivity of AR MAb with a 90 to 110 kD protein. Preputial cells in culture, therefore, have the potential to be regulated by both steroid and nonsteroid hormones as demonstrated by unique biochemical and immunological markers. This sebaceous cell system may be used to study the mechanisms of action and interaction of steroid (androgen) and nonsteroid (catecholamine) hormones in disorders of sebaceous cells such as acne.

THE EFFECT OF A NEW ANTIMICROBIAL PEPTIDE MAGAININ ON *PSEUDOMONAS AERUGINOSA* PROLIFERATION IN WOUNDS. P.M. Mertz, D.A. Marshall, A.L. Cazzaniga, V. Wassertiel, W.H. Eaglstein. Univ. of Miami Sch. of Med., Dept. Derm., Miami, Fl and B. Berkowitz, Magainin Sciences Inc., Plymouth Meeting, PA.

To study *in vivo* the antimicrobial effects of topically applied Magainin, a unique antimicrobial peptide isolated from frog skin, 72 partial thickness wounds were made on the back of 2 young pigs. Each wound was inoculated with 10^4 *Pseudomonas aeruginosa* to establish a local infection. Wounds were treated topically b.i.d. with either no treatment, cream vehicle, Magainin 0.1% or 0.25%, erythromycin, or a combination of Magainin and erythromycin. Wounds were cultured after 24 and 48 hours by the scrub technique to determine the number of *P. aeruginosa* present. Differences were seen at 48 hours when wounds were treated with 0.25% Magainin. Results are seen in the table below:

Recovery of *P. aeruginosa* (48 hours)	(CFU/ml ± S.D.)
Untreated	4.1 ± 0.5
Vehicle	3.3 ± 0.8
Magainin	1.4 ± 0.0
Erythromycin	4.2 ± 0.7
Magainin and erythromycin	3.0 ± 2.6

These preliminary studies suggest topical Magainin has a strong *in vivo* anti-pseudomonal activity.

CELLULAR HETEROGENEITY IN DYSPLASTIC NEVI AND HUMAN MELANOMAS. Estela Medrano, Jamal Farooqui, Raymond Boissy, James Nordlund, Dept of Dermatology, Univ of Cinti Med Center, Cincinnati, OH.

Cellular heterogeneity, although neglected for many years, is now recognized as one reason for failure of chemotherapy and radiation for human tumors. To improve our knowledge of the developmental biology of human melanomas, we have established long term *in vitro* growth conditions for dysplastic nevi (DN) (Ca^{++} free MEM, 10% FCS, 5 μg/ml insulin, 5 ng/ml EGF, 40 mM TPA, 5 mM CT and 1 μg/ml α-tocopherol) and adult melanocytes (MCDB medium supplemented with 5% FCS, 0.3 ng/ml bFGF, growth factors and α-tocopherol as above, and 4 ng/ml TPA.) DN were characterized by electron microscopy, growth curves and expression of fibronectin by immunocytochemistry. By using the silica colloid technique (Percoll) recently described (Resnicoff M, Medrano EE, et al. Proc Natl Acad Sci USA 84:7295-7299, 1987), we now show that dysplastic nevi and melanomas can be separated in subpopulations which show different growth characteristics and antigen expression. Established uncloned melanoma cell lines show Percoll profiles invariant in time. DN cells eventually evolve toward senescense after more than 100 doublings. A "senescent profile" is characterized by a shift to the densest fraction of the gradient with highly pigmented cells. A strong social behavior in DN is illustrated by the different growth capacities shown by the subpopulations when they are grown isolated. This model may be useful to study cellular interactions, growth factor requirements, tumor progression and response to drugs in the human melanocytic system.

PHARMACOLOGICAL INTERACTIONS BETWEEN GLUCOCORTICOIDS AND RETINOIDS IN MOUSE SKIN. J.A. Mezick, G.J. Gendimenico, A.J. Perlin, M.E. Rosenthale, R.J. Capetola, R.W. Johnson Pharmaceutical Research Institute at Ortho Pharmaceutical Corp., Raritan, NJ.

All-trans-retinoic acid (RA) prevents dexamethasone-induced skin atrophy in hairless mice. The purpose of these studies was to determine 1) if RA prevents the skin atrophy induced by other potent glucocorticoids, 2) if retinoids structurally different from RA prevent glucocorticoid-induced skin atrophy in hairless mice, 3) if RA interferes with antiinflammatory activity of glucocorticoids and 4) if glucocorticoids interfere with the biological activity of RA. For skin atrophy, female Skh-1 hairless mouse dorsal skin was treated with glucocorticoids in the a.m. and retinoids in the p.m., once daily, 5 days/week for 2 weeks. Atrophy was assessed by skin-fold thickness and ultrasound A-scan. Antiinflammatory effects of dexamethasone (DEX) without and with RA were tested in the oxazolone-induced contact hypersensitivity (Ox-CHS) model in CD-1 mice. Biological activity of RA was assessed by reduction of horn-filled utriculi in rhino mouse skin. RA (0.05%) prevented the skin atrophy induced by 0.05% clobetasol propionate (CP), 0.5% triamcinolone acetonide and 0.05% fluocinolone acetonide. Four retinoids, having widely different potencies, prevented atrophy induced by 0.05% CP. The dose-related antiinflammatory effect of DEX (0.001%-0.1%) in the Ox-CHS model was not affected by pretreatment with 0.025% RA. The ability of RA (0.0001%-0.01%) to cause a dose-related reduction in the size of rhino mouse utriculi was unaffected by treatment with 0.05% CP. These results show retinoids prevent skin atrophy without affecting the antiinflammatory effect of glucocorticoids, and the biological activity of retinoids is not prevented by glucocorticoid treatment. Thus, retinoids prevent glucocorticoid-induced skin atrophy without a loss of therapeutic effects of glucocorticoids or retinoids.

SEASONAL VARIATION IN BLOOD PRESSURE IN SYSTEMIC SCLERODERMA. Peter B. Milburn, Joseph Onorato, Joyce Z. Singer. Departments of Dermatology and Medicine (Division of Rheumatology), SUNY Health Science Center, Brooklyn, New York.

Records of 26 patients with systemic scleroderma were reviewed to determine whether variation in blood pressure occurred between summer and winter months. The means of summer blood pressure values in these patients were 119.7 mm Hg.(systolic), and 75.4 mm Hg.(diastolic); the means of winter blood pressure values were 130.8 mm Hg.(systolic), and 81.3 mm Hg.(diastolic). The differences were highly significant ($p<0.001$). Statistically significant differences were also seen in subgroups of scleroderma patients on no anti-hypertensive medications [$p<.025$ (systolic), $p<.01$ (diastolic)], as well as in scleroderma patients receiving therapy for hypertension [$p<.001$ (systolic), $p<.05$ (diastolic)]. No statistically significant seasonal variation was seen in the blood pressure measurements of normotensive or hypertensive control patients with either rheumatoid arthritis or osteoarthritis. These findings indicate a significant variation in blood pressure between the summer and winter months, which may result from vasoconstriction induced by chronic exposure to cold weather. This variation may have relevance to the development of scleroderma renal crisis, pulmonary disease, and digital ulcerations.

THE SIGNIFICANCE OF SINGLET OXYGEN AND DNA CROSS-LINKS IN SKIN PHOTOSENSITIZATION BY TRICYCLIC AND TETRACYCLIC PSORALENS. S. Mobilio, M. Palumbo, and M.A. Pathak, Department of Dermatology, Harvard Medical School, Boston, MA.

Although skin photosensitization (erythema, edema, blistering) by psoralens (e.g., 8-MOP) is attributed to their ability to cross-linking double-stranded DNA, evidence presented here indicates singlet O_2 ($^1O_2$) contributes strongly to skin photosensitization. Using four tetracyclic psoralens (benzopsoralens and cycloesenylic psoralens) having an additional aromatic or cycloesenylic ring condensed to the furan ring of tricyclic psoralens (e.g., 8-MOP, psoralen), we carried out a comparative study to determine: (a) their photosensitizing potency in guinea pig skin, (b) their dark interaction with DNA, (c) DNA photobinding involving the formation of monofunctional and bifunctional adducts (single strand adducts and cross-links), and (d) $^1O_2$ generating capacity by methods previously reported. Tetracyclic benzopsoralens showed greater non-covalent binding to DNA than cycloesenylic psoralens. Both photoreacted more strongly with pyrimidine bases in DNA than 8-MOP or 5-MOP. Benzopsoralens produced monofunctional adducts whereas 8-MOP or 5-MOP revealed strong DNA cross-linking ability and the cycloesenylic psoralens exhibited a weak ability to cross-link DNA. The tetracyclic 4-methyl, tetrahydrobenzopsoralen was a strong skin photosensitizing agent similar to 8-MOP. The benzopsoralens and tricyclic psoralen in general exhibited greater ability to produce $^1O_2$ than non-phototoxic cycloesenylic psoralens. The role of oxygen is significant in skin photosensitization by psoralens.

MOLECULAR CHARACTERIZATION OF INTERACTIONS BETWEEN ALL-TRANS-RETINOIC ACID (RA), GAMMA INTERFERON (IFN-γ) AND CULTURED HUMAN KERATINOCYTES. R.S. Mitra, G. Karabin, J. Barker, J. Varani, J.J. Voorhees, B.J. Nickoloff, Departments of Pathology and Dermatology, Univ of Michigan, Ann Arbor, MI

Retinoids have therapeutic effects for a wide variety of skin disorders including psoriasis and photoaged skin. However, the molecular basis for retinoid induced effects on the epidermis, or possible interaction between retinoids, and products of activated T cells (i.e. IFN-γ), which accompany many skin diseases is not well understood.

For proliferation assays, $6x10^4$ KCs were seeded in 24-wells and allowed to grow in the dark in a low-calcium, serum-free medium for 48 hrs either with RA (1 ug/ml) alone, IFN-γ (100 U/ml) alone, or RA plus IFN-γ. Cell counts revealed that control, KCs underwent 2 population doublings. For ligand-binding studies, 48 hr treated KCs ($2x10^5$ cells/well) were prewashed with EGF free basal medium for 2 hrs at 37°C, followed by addition of ^{125}I-EGF (0.032 to 3.2 nM) at 4°C for 6 hrs. TGF-α protein secretion was measured using a R.I.A. kit (Biotope). Immunoprecipitation of EGF receptor (EGFr) utilized anti-EGFr antibody (ICN Biomedical). Northern blot analysis was performed by routine procedures.

RA directly stimulated KC growth at 48 hrs by 140 +/-12% of control cultures (N=4; p<.01); whereas IFN-γ directly inhibited KC growth by 50% of control (N=4; p<.01). Moreover, when RA + IFN-γ were combined, the RA antagonized the IFN-γ effect, since combined cell cultures grew at approximately the same rate as untreated cultures (106%; N=4). Control KCs had high affinity EGFr calculated by Scatchard analysis as: $Kd=0.86x10^{-9}$M; 94,000 sites/cell. 48 hrs after RA exposure there were only 37,000 sites/cell; and with IFN-γ there were 48,000 sites/cell. Neither treatment influenced the Kd. TGF-α production at 48 hrs by treated KCs was increased by approximately 50% by RA (17 ng/ml/10^5 KCs). In contrast to markedly diminished ligand binding results at 48 hrs, there was no detectable change in amount of immunoprecipitable EGFr induced by either RA or IFN-γ, suggesting functional alteration in ligand binding ability of EGFr, rather than purely quantitative effects. The EGFr mRNA increased 2 fold by RA treatment at 6 hrs, whereas the EGFr mRNA was decreased by 50% after IFN-γ exposure for 6 hrs; this decline was abrogated by the co-presence of RA in the short term (6 hrs). EGFr mRNA was completely undetectable after 48 hrs of combined treatment.

We propose that the clinical effects of RA on photoaged skin, in which there is rapidly induced epidermal hyperplasia, is mediated by increasing KC EGFr mRNA and TGF-α production; whereas the delayed therapeutic effects of RA on psoriatic lesions is mediated by chronic and persistent decreased EGFr mRNA which occurs in the co-presence of IFN-γ.

GAMMA/DELTA T-LYMPHOCYTES IN MYCOBACTERIAL INFECTION. R. Modlin, P. Barnes, K. Uyemura, and T. Rea, USC School of Medicine, Los Angeles, CA.

T-lymphocytes bearing the γδ antigen receptor (TCR) have been reported to be a component of the immune response to mycobacteria. TCRγδ cells were quantitated in patients with mycobacterial disease after stimulation with antigen and expansion with IL-2. M. tuberculosis (H37RA strain) stimulation of blood increased TCRγδ cells (TCRδ1+) from 4% to 19% in tuberculosis (TB) contacts (n=10) but from 2% to 4% in TB patients (n=10). Cells from DTH skin tests of tuberculoid leprosy patients were expanded with M. leprae to be >15% δ+ in 4/5 lesions but <3% in 5 blood lines. δ+ lines (n=2) of >90% purity from leprosy patients and 7/8 from TB contacts proliferated specifically to mycobacterial antigen with stimulation indices >10. The diversity of the TCRγδ repertoire was estimated by analysis with antibodies to Vδ1+ (TCSδ1) and Vδ2 (BB3). In 4 lines from skin tests 50-100% of δ+ cells were Vδ1+ with 3-17% Vδ2+, but in 10 lines from TB contact blood 3/10 were predominantly expressing Vδ2 (76-100%). The findings suggest that antigen reactive γδ cells are present in the blood of patients with resistance to TB infection and in the DTH reactions of patients with limited disease. The use of multiple V region gene segments suggest that γδ T-cells in mycobacterial disease have a complex repertoire of antigen recognition.

MELANOCYTES PRODUCE IL-1 BETA AND CONTAIN AN IL-1 BETA CONVERTASE ACTIVITY: A POTENTIAL IN VIVO MECHANISM FOR PARACRINE CONVERSION OF KERATINOCYTE PRO-IL-1 BETA. H. Mizutani, N. Miwa, T. Mizutani, and T.S. Kupper, Washington and Yale Univ., St. Louis MO/New Haven CT.

Melanoma cells and cultured melanocytes contain mRNA's for both IL-1 alpha and beta. However, we have demonstrated that IL-1 mRNA expression correlates poorly with protein production and biological activity. We therefore performed Western blot analysis on cultured melanocytes using monoclonal antibodies to both IL-1 alpha and beta. Melanocytes contain both IL-1 alpha and beta proteins. Although IL-1 alpha is biologically active in its precursor (33 kD) and processed (17 kD) forms, 33 kD IL-1 beta is biologically inactive and requires conversion to a 17 kD molecule for activity. This is mediated by a newly described enzyme called monocyte IL-1 convertase, previously thought to be lineage specific for myelomonocytic cells. Since melanocytes produced both 33 and 17 kD IL-1 beta, we asked whether melanocytes contained a similar convertase activity. 33 kD IL-1 beta was incubated with lysates of cultured melanocytes, monocytes (convertase positive), and keratinocytes (convertase negative). Western analysis showed that melanocyte lysates converted 33 kD to 17 kD IL-1 beta; the mobility of the 17 kD species was identical to that of the monocyte convertase derived species. Keratinocyte lysates did not convert 33 kD IL-1 beta. This is the first evidence that a non-leukocytic cell contains an IL-1 beta convertase, and raises the possibility that melanocyte IL-1 beta convertase may act on 33 kD produced by convertase-negative keratinocytes to generate an active product.

THE EFFECT OF ISOQUINOLINESULFONAMIDES (H7,H8) ON LTC_4-INDUCED MELANOCYTE GROWTH AND PIGMENTATION. J.G. Morelli, S.S.Hake, R.C. Murphy, D.A. Norris. Departments of Dermatology and Pharmacology, UCSM, Denver, CO.

Leukotriene C_4 (LTC_4) is a potent stimulant for melanocyte growth. LTC_4-induced melanocyte growth is accompanied by a decrease in the amount of pigment per individual melanocyte, yet the mechanism of action of LTC_4-induced effects on melanocytes is unknown. We examined the effects of the protein kinase C inhibitor(H7) and the cyclic nucleotide dependent kinase inhibitor(H8) on these processes. Melanocytes were obtained from human neonatal foreskins. Melanocytes were isolated after 7-14 days in co-culture with keratinocytes in Keratinocyte Growth Media(KGM). Melanocytes were then grown for one week in either KGM alone or KGM plus LTC_4 100nM with or without H7(n=5) or H8(n=5)(25-100uM). Melanocytes were counted and melanin content determined spectrophotometrically. The addition of H7 significantly increased LTC_4-induced melanocyte growth($p<.030$) while further enhancing the decrease in pigmentation per melanocyte($p<.014$). Conversely, the addition of H8 did just the opposite, inhibiting growth($p<.001$) and enhancing pigmention per melanocyte($p<.010$). LTC_4-induced melanocyte growth is cyclic nucleotide dependent, whereas pigment production is PKC dependent. We believe physiologic stimulation of human melanocyte growth and pigmentation are mediated through separate pathways.

PROLIFERATING CELLS IN PSORIATIC DERMIS ARE COMPOSED PRIMARILY OF T CELLS, ENDOTHELIAL CELLS, AND FACTOR XIIIa$^+$ PERIVASCULAR DENDRITIC CELLS. Greg S. Morganroth, Lawrence S. Chan, James D. Walter, John J. Voorhees, Gerald D. Weinstein*, and Kevin D. Cooper, Departments of Dermatology, the Univ. of Michigan, Ann Arbor, Michigan, and the Univ. of California, Irvine, California*.

Determination of the cell types proliferating in the dermis of patients with psoriasis should identify those cells experiencing activation or responding to growth factors in the psoriatic dermal milieu. Toward that end, sections of formalin-fixed ^{3}H-deoxyuridine (^{3}H-dU) injected biopsies of involved and uninvolved skin from five patients were immunostained, followed by autoradiography. Proliferating dermal cells exhibit silver grains from tritium emissions. The identity of the proliferating cells could then be determined by simultaneous visualization of antibodies specific for various cell types. UCHL1$^+$ (CD45$^+$) T cells (recall antigen-reactive helper T cell subset) constituted 39.8±2.9% (mean±SEM, n=5) of the proliferating dermal cells in involved skin, while Leu 18$^+$ (CD45R$^+$) T cells (naive, suppressor/inducer and cytotoxic/suppressor T cell subsets) comprised only 5.3±1.4% (n=5). Factor XIIIa$^+$ dermal dendritic leukocytes (26.6±3.1% of proliferating dermal cells, n=5) and Factor VIII$^+$ endothelial cells (20.7±4.5%, n=3) represented the two other major proliferating populations in lesional psoriatic dermis. The density of these cell types exhibiting proliferation was also increased in involved vs. uninvolved dermis (UCHL1$^+$: 9.0±1.9 vs. 2.5±0.2 cells/mm^2, p<.05; Factor XIIIa$^+$: 6.4 ±1.2 vs. 1.6±0.3 proliferating cells/mm^2, p<.05; Factor VIII$^+$: 7.0±1.1 vs. 0.0 proliferating cells/mm^2, p<.05). Melanophages, as well as Leu M1$^+$ (CD15$^+$) and Mycloid Histiocyte Antigen$^+$ macrophages and granulocytes comprised less than 5% of the proliferating cells. The presence of an actively proliferating T cell subset in lesional dermis suggests that specific activating signals are contained within psoriatic dermis in vivo. The activation of recall antigen-reactive T cells may be a driving force behind the dendritic leukocyte and endothelial cell proliferation. Alternatively, the selective proliferation and expansion of these two constitutive cell types may result in signals that promote activation of UCHL1$^+$ T cells.

GLUTATHIONE S-TRANSFERASE CATALYZED METABOLISM OF LEUKOTRIENE A_4 TO LEUKOTRIENE C_4 IN RODENT AND HUMAN SKIN. Hasan Mukhtar, Haider Raza, David L. Allyn, and David R. Bickers, Dept. Dermatology, Case Western Res. Univ. and VAMC, Cleveland, OH.

Leukotrienes constitute a group of oxygenated metabolites of arachidonic acid with biological activities related to hypersensitivity and inflammation. Biosynthesis of these compounds proceeds via the unstable intermediate leukotriene A_4 (LTA_4) catalyzed by the 5-lipoxygenase pathway. Conjugation of LTA_4 with reduced glutathione (GSH) catalyzed by glutathione S-transferases (GSTs) leads to the formation of the vasoactive leukotriene C_4 (LTC_4). The skin is a major site of inflammation and yet cutaneous metabolism of LTA_4 to LTC_4 has not been studied. In this study we investigated the GST-dependent conjugation of GSH with LTA_4 in rodent and human skin. Incubation of ^{3}H-LTA_4-methyl ester (1 nmole, 200,000 DPM) with cytosol prepared from rat, mouse and human skin in the presence of GSH resulted in the formation of LTC_4. Product formation was identified by its UV absorption spectrum and radioactivity profile on HPLC. With heat denatured cytosol or in the absence of GSH LTC_4 formation was negligible. Maximum activity occurred in rat skin followed by mouse and human. LTA_4-GST activity was also measured in keratomed epidermis from normal individuals and from lesional and nonlesional skin of patients with psoriasis. Enzyme activity in involved psoriatic plaques (24.13 ± 1.86 pmole/min/mg protein, n=3) was significantly increased when compared with uninvolved (11.32 ± 2.18, n=3) and normal (5.97 ± 1.17, n=8) epidermal biopsies. These results suggest that LTA_4 is biotransformed to LTC_4 by a GST catalyzed reaction in rodent and human skin and that this activity is significantly increased in psoriasis.

ACTIVATED HUMAN LANGERHANS CELLS CONTAIN mRNA FOR PROOPIOMELANOCORTIN. V.B. Morhenn, K. Stage, and S. Lee. Dept. of Dermatology UC Davis, Davis, CA and Syntex Corp., Palo Alto, CA.

Proopiomelanocortin (POMC) gene expression has recently been reported in murine epidermal cells. The epidermis is comprised of several cell types including keratinocytes (KC) and Langerhans cells (LC). We determined whether either of these 2 cell types has the capacity to express POMC mRNA in human epidermis.

Epidermal cell suspensions were enriched for LC and KC by panning with monoclonal antibody against CD-1. The LC and KC fractions were incubated overnight with or without phorbol myristate acetate (PMA) (10^{-7}M) and /or lipopolysaccharide (LPS 20 µg/ml). The mRNA was extracted and probed with radioactive cDNA for POMC. Untreated KC and LC fractions showed very little or no expression of the POMC mRNA. The treated KC fraction also showed no POMC mRNA. By contrast, the LC fraction exposed to PMA or PMA plus LPS demonstrated POMC mRNA. LPS alone did not induce POMC mRNA expression.

Since POMC mRNA encodes the precursor(s) for various proteins including β-endorphin and melanocyte stimulating hormone (MSH), these data suggest that β-endorphin and MSH could be secreted in the skin after appropriate stimuli. Experiments are currently in progress to characterize further the factors involved in the regulation of POMC mRNA expression.

A COMPARISON OF THE INHIBITORY POTENTIAL OF MINOXIDIL AND ITS STRUCTURAL ANALOGS TOWARD LYSYL HYDROXYLASE IN CULTURED FIBROBLASTS. Saood Murad, Linda C. Walker, Garland A. Johnson, and Sheldon R. Pinnell, Division of Dermatology, Department of Medicine, Duke University Medical Center, Durham, North Carolina and Hairgrowth Research, The Upjohn Company, Kalamazoo, Michigan.

Minoxidil, 2,6-diamino-4[1-piperidinyl]pyrimidine-1-N-oxide, has been shown to inhibit the expression of lysyl hydroxylase in cultured fibroblasts, leading to the synthesis of a collagen specifically deficient in hydroxylysine. Cultures of human skin fibroblasts were incubated for 72 hr with 1 mM minoxidil or one of its structural analogs. Lysyl hydroxylase activity (% of control) in extracts of treated cells was as follows: minoxidil (19-39%); compound I - methyl group in position 2 (32%); compound II - methyl group in position 6 (37%); compound III - methyl group in both positions 2 and 6 (111%); compound IV - methylamino group in position 4 (104%); compound V - diethylamino group in position 4 (52%); compound VI - hexylamino group in position 4 (93%); compound VII - pyridine ring replacing the pyrimidine ring (31%); compound VIII - symmetrical triazine ring replacing the pyrimidine ring (41%); compound IX - morpholine ring replacing the piperidine ring (95%). The results indicate that only one of the two amino groups of minoxidil is essential for its inhibitory property. The pyrimidine ring can be replaced with a pyridine or triazine ring without loss of inhibitory activity and the piperidine ring can be replaced with a dialkylamino group with some loss of inhibitory activity.

DISSOCIATION OF INOSITOL LIPID HYDROLYSIS FROM CELL PROLIFERATION FOLLOWING TREATMENT WITH GROWTH FACTORS AND BRADYKININ IN HUMAN KERATINOCYTES. Vera B. Morhenn, Kathleen L. King and Randolph M. Johnson. Dept. of Dermatology, Univ. of California, Davis and Dept. of Developmental Biology, Genentech, Inc., So. San Francisco, CA.

The early transmembrane signalling events regulating growth of keratinocytes (KC) remain obscure. Inositol (Ins) lipid hydrolysis is currently thought to play a role in growth factor-induced cellular proliferation. Since human epidermal growth factor (hEGF) and human transforming growth factor-α (TGF-α) are known to stimulate KC growth, we compared the ability of these two proteins with bradykinin (BK) to stimulate Ins polyphosphate and *sn*-1,2-diacylglycerol (DAG) formation in KC. KC were grown in low calcium, serum-free medium (KGM, Clonetics, Inc.) plus bovine pituitary extract. KC were labeled with 20 µCi/ml [^{3}H]-*myo*-inositol in basal medium (KBM, Clonetics) plus insulin and hydrocortisone for 24 h. After labeling, KBM containing 10 mM Li^+ was added for 20 min. Cells were incubated for various times with hEGF (200 ng/ml), hTGF-α (200 ng/ml) or BK (0.1 µM) and the soluble Ins polyphosphates were resolved by HPLC. DAG content was determined using the DAG kinase reaction. Consistent with previous reports, BK stimulated Ins(1,4,5)P_3 formation 2.9-fold over control by 60 s. There were concomittant increases in Ins(1,3,4)P_3, Ins(1,4)P_2 and Ins(1)P. BK-stimulated DAG rose from 143 to 211 pmol/plate in 60 s. In contrast, hEGF and hTGF-α did not elevate Ins(1,4,5)P_3 or other isomers of Ins polyphosphates by 2 min nor did they significantly increase DAG levels after 2 min (basal 143, hEGF 144, hTGF-α 150 pmol/plate). In addition, BK inhibited growth of KC cultures treated for 6 days. These results suggest that in this cell system stimulation of Ins lipid hydrolysis does not necessarily correlate with cell proliferation. Other second messengers that putatively are involved in the growth response to hEGF and hTGF-α are under investigation.

CHRONIC, NON-HEALING SKIN ULCERS DISPLAY A GROWTH-ACTIVATED EPIDERMIS (REGENERATIVE MATURATION) WITH HIGH LEVEL TGF-ALPHA AND EGF-RECEPTOR EXPRESSION. D Murphy, B Smoller, AB Gottlieb, A Hsu, DM Carter, and JG Krueger, Rockefeller Univ. and Cornell Univ. Med. College, NY, NY 10021

Treatment of chronic skin wounds is hampered by lack of understanding about specific cellular and molecular pathology in this condition. Lack of appropriate growth factors is frequently hypothesized as a cause of non-healing wounds. Because TGF-alpha and other epithelial cell growth factors are produced in an autocrine or paracrine fashion from keratinocytes, we examined chronic, non-healing ulcers on the lower extremities of 15 patients for the presence of TGF-alpha, EGF-receptors, and for a growth-activated epidermis. A transient epidermal phenotype of regenerative maturation occurs in acute skin wounds and is defined by histochemical staining of suprabasal keratinocytes with filaggrin, involucrin, keratin (AE1), and psi-3 antibodies. Fifteen of 15 chronic wounds showed marked regenerative maturation (growth activation) in epidermis at the wound edge, comparable to regenerative maturation in acute skin wounds. Control sections of normal skin obtained from each of these individuals showed a normal, resting epidermal phenotype with each of these antibodies. Compared to normal skin, epidermis adjacent to chronic wounds showed overexpression of cell-associated TGF-alpha and of EGF-receptors as detected with specific monoclonal antibodies to each of these proteins. High-level expression of EGF-receptors and of TGF-alpha was similar to that detected in active psoriatic skin. Thus, epidermal proliferation in skin wounds could be regulated in an autocrine fashion, as proposed in psoriatic epidermal hyperplasia. These findings have clear implications for pathogenesis and treatment of chronic wounds.

RELEASE OF TRANSFORMING GROWTH FACTOR-ALPHA BY ULTRAVIOLET RADIATION. TIME COURSE AND ACTION SPECTRUM STUDIES. G.M.Murphy, D.G.Quinn, R.D.R.Camp, J.L.M.Hawk, M.W.Greaves, The Institute of Dermatology, St Thomas's Hospital, London, UK.

Transforming growth factor-alpha (TGF-alpha) is produced by and required for growth of epithelial cells. It is present in normal human epidermis and TGF-alpha over-production may be responsible for epidermal hyperproliferation in psoriasis. Ultraviolet (UV) B irradiation of human skin leads to epidermal damage and subsequent hyperplasia, whereas UVA irradiation has predominantly dermal effects. We studied the relative abilities of UVB and UVA to release TGF-alpha and its time course. Ten subjects of skin Type I and II were irradiated on the back on non-sun exposed skin with three times the minimal erythema dose of UVB (TL20/W12 lamps) and UVA (Uvasun 2000). Using the skin chamber technique, exudate samples were obtained from irradiated sites immediately and 4 and 24 h after irradiation and from non-irradiated adjacent skin. Exudate was collected 30 min after chamber application, snap frozen in liquid nitrogen and assayed using a specific TGF-alpha radioimmunoassay. Significantly elevated levels of immunoreactive TGF-alpha (nmoles/l) were found in 24 h UVB but not UVA irradiated compared with non-irradiated skin. (24 h UVB: 15.7 $\pm$ 3.4 S.D.; unirradiated: 11.2 $\pm$ 5.02; $P < 0.001$, Paired t test). Samples at all time points from non-irradiated and from UVB and UVA exposed skin contained measurable levels of TGF-alpha, possibly due to the trauma of sampling. These results suggest that UVB-evoked TGF-alpha may stimulate epidermal hyperplasia in irradiated skin.

MAJOR HISTOCOMPATIBILITY COMPLEX MARKERS IN JAPANESE PATIENTS WITH PSORIASIS VULGARIS. Hidemi Nakagawa, Shuichi Akazaki, Katsushi Tokunaga, Kazumasa Matsuki, Yasumasa Ishibashi, and Takeo Juji, Department of Dermatology and Blood Transfusion Service, Faculty of Medicine, Tokyo Univ., Tokyo, Japan.

Genetic polymorphisms of HLA antigens and HLA-linked serum complement components (C2, C4A, C4B, and BF) were investigated in 56 Japanese patients with psoriasis vulgaris. HLA typing revealed increased frequencies of HLA-A1, A2, B39, Bw46, Cw6, Cw7 and Cw11. HLA-Bw46 and Cw11 showed particularly strong associations. Among complement components, positive associations were obtained with C4A4 and C4B2 and a negative one with BFF. The major histocompatibility complex haplotype (supratype), HLA-A2-Cw11-C2C-BFS-C4A4-C4B2-DRw8 is purported to be a new high risk haplotype in Japanese patients with psoriais vulgaris. Analysis of patients with this supratype via pulsed field gel electrophoresis showed existence of specific, extensive DNA deletions near HLA DR genes, but no disease-specific patterns could be observed by means of this technique. The newly found HLA associations and the high risk haplotype indicate racial and ethnic differences among psoriatic patients.

EXPERIMENTAL MODEL FOR THE ANALYSIS OF AUTOIMMUNITY IN PROGRESSIVE SYSTEMIC SCLEROSIS. Tai Muryoi, Kuppuswamy N. Kasturi, Jerome S. Perlish*, Raul Fleischmajer*, and Constantin A. Bona, Department of Microbiology and *Department of Dermatology, Mount Sinai School of Medicine, New York, New York.

Tight skin mice develop cutaneous hyperplasia and histopathological alterations of skin similar to those described in diffuse type scleroderma. These mice also produce anti-topoisomerase I specific autoantibodies, characteristically present in the sera of a subpopulation of the scleroderma patients. However, the etiology of the human disease is unknown. From unimmunized tight skin mice spleen cells we have isolated a number of hybridomas secreting monoclonal antibodies specific for topoisomerase I. We have examined the immunochemical and functional properties of these antibodies and compared them with that of scleroderma patients. Results of Western blot analysis and competitive inhibition in RIA indicate that mouse anti-topoisomerase I antibodies recognize the same or similar epitopes recognized by autoantibodies found in scleroderma patients. Furthermore, one monoclonal antibody (34A1-26) also neutralizes the enzymatic activity of topoisomerase I similar to autoantibodies present in scleroderma. These results suggest that the autoantibodies present in scleroderma patients and tight skin mice recognize some conserved epitopes present on the topoisomerase I polypeptide which confer the catalytic property for the enzyme.

INCREASED EPIDERMAL GROWTH FACTOR RECEPTOR IN SEBORRHEIC KERATOSES AND ACROCHORDONS IN PREGNANT WOMEN WITH DYSPLASTIC NEVUS SYNDROME. L.B. Nanney, D.L. Ellis, S.B. Dorsey, L.E. King, Depts. of Plastic Surgery and Cell Biology, Dermatology, Vanderbilt & V.A. Medical Centers, Nashville, TN

To determine the effects of sex steroid hormones and Dysplastic Nevus Syndrome (DNS) on EGF-R metabolism, EGF-R localization patterns were compared in lesions from women with or without DNS who were pregnant or taking oral sex steroid hormones to lesions from matched control groups. Growth effects on EGF-R in seborrheic keratoses and acrochordons were studied by comparing the growth history of lesions to their EGF-R immunostaining pattern. Urinary TGFα was determined by RIA in selected patients. EGF-R concentrations were elevated in suprabasilar keratinocytes of actively growing lesions from DNS patients who were pregnant or taking sex steroid hormones. Elevated levels of EGF-R were also seen in growing seborrheic keratoses from normal or control DNS patients. In contrast, EGF-R distribution patterns in acrochordons did not correlate well with lesional growth histories in normal and control DNS patients. Although the etiology of increased levels of EGF-R in these hyperproliferative cutaneous lesions is unknown, the EGF-R distribution suggests a possible mechanism for EGF/TGF-α in the evolution of these skin lesions.

EFFECT OF SINGLE UVR EXPOSURE ON SKIN PIGMENTATION AND MELANOCYTE MORPHOLOGY IN THE YUCATAN MINIATURE SWINE. X. Nair, Ph.D. and K. M. Tramposch, Ph.D. Dermatology Research, Bristol-Myers Squibb, Co., Pharmaceutical R&D Division, Buffalo, NY 14213.

The effect of a single ultraviolet (UVR) radiation on skin pigmentation and melanocyte morphology and numbers was investigated in the Yucatan miniature swine, a specie which share several skin characteristics with humans. A 150 watt Xenon arc solar simulator lamp (295-400nm) with a 1 mm Schott WG 320 filter was used. Under sedation the flank region of the swine was exposed to different UVR which varied from a suberythemal dose to 1-2 MEDs. The change in skin pigmentation was graded on a 0 (normal) to 4 (dark brown) scale. Skin biopsies were taken at 3, 7, 14, 21 and 49 days after UVR. The number of melanocytes was determined using L-dopa-incubated split epidermal preparations. Vertical sections of whole skin stained with Fontana-Masson stain were also examined for change in melanocyte density. Dose related increase in pigmentation of the exposed skin was apparent by Day 7 after UVR and peaked (pigment grade 2-4) between Days 21 and 28. Microscopically, the melanocytes demonstrated increased numbers of dendrites 3 days after UVR. A dose-related and time-dependent increase in the number of dopa-positive melanocytes with enlarged perikaraya and dendrites was apparent by Day 7. The vertical sections also showed a similar pattern of increase in the dopa stained split skin sections. The onset of visible hyperpigmentation lagged behind the increase in melanocyte numbers and size. Hence a single exposure of UVR produced a marked increase in enlarged melanocytes with enlarged dendrites with a corresponding increase in visible skin pigmentation.

IMMUNOHISTOCHEMICAL DEMONSTRATION OF NERVE-MERKEL CELL COMPLEX IN HUMAN SKIN DEVELOPMENT. Yutaka Narisawa, Ken Hashimoto, Masahiko Ishihara, and Dwayne Lawrence, Department of Dermatology(YN, KH, MI) and Pathology(DL), Wayne State Univ. School of Medicine, Detroit, MI and VAMC, Allen Park, MI

The origin of human epidermal Merkel cell has been controversial. The neural crest theory is primarily based upon the occurrence of dermal, nerve-associated Merkel cells in human fetal skin. In this study we investigated the formation of nerve-Merkel cell complex in sequential stages of skin development. Availability of Merkel cell specific antikeratin monoclonal antibodies and antineurofilament antibody made it possible to study nerve-Merkel cell relationship. We used a double staining method on frozen sections. Merkel cells were stained with monoclonal anti-Cytokeratin CK5(Sigma,St.Louis,MO) which reacts against human cytokeratin polypeptide 18. On the other hand, peripheral nerves were stained with monoclonal murine antibody Neurofilament 70 and 200 Kd(SIGNET,Dedham,MA). In the epidermis and dermis of plantar skin of human embryo, epidermal Merkel cells were recognized after 12 weeks of gestation. Dermal Merkel cells were recognized only after 17 weeks of gestation. Immunoreactive small nerves reached the epidermis after 17 weeks of gestation and nerve-Merkel cell complex was found in specimens older than 16 weeks of gestation; in other words, the appearance of epidermal Merkel cells preceded the attachment of immunoreactive small nerves to the epidermis. It was concluded that Merkel cells do not arrive at the epidermis with peripheral nerve and that they may very well be originated in the fetal epidermis.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF MAST CELL CARBOXYPEPTIDASE: CORRELATION OF SEQUENCE AND SUBSTRATE PREFERENCE. M. Natsuaki, C-B Stewart#, C.E. Kaempfer, P. Vanderslice*, M.S. Natsuaki, L.B. Schwartz**, B.U. Wintroub, and S.M. Goldstein, Dept. of Derm; Hormone Res. Inst#; CVRI*; U.C. San Francisco, and Depts of Micro., Immunol, and Path, Med Coll of VA**

Mast cell (MC) carboxypeptidase (CP) is a unique CP recently purified from skin. N-terminal amino acid (AA) sequencing revealed that MCCP and pancreatic CPs are homologous enzymes. It is thought that MCCP is stored in MC granules as an active enzyme complexed with heparin proteoglycans, and not as a proenzyme. We report studies that further characterize this enzyme. Using an 5' oligonucleotide, a 500 bp partial cDNA clone for MCCP corresponding to a ~90 AA procarboxypeptidase sequence was isolated from a human lung MC cDNA library. This suggests that MCCP, like pancreatic CPs, may be 1st translated as a proenzyme. cDNA sequencing shows limited positional identity between MCCP and CPA and CPB. Using this cDNA, 3 additional clones of 2, 1.6, and 0.8 kb were isolated from a human skin MC library.

In addition, isolation and AA sequencing of a leucine aminopeptidase fragment of purified MCCP containing the S_1' binding site reveals 3 AA substitutions compared to bovine CP. To determine the correlation between the structure of the MCCP active site and its activity, Km/kcat of MCCP and bovine pancreatic CPA was determined for a series of tripeptide substrates (Cbz-Gly-Gly-X) substituted at the C-terminal AA. Bovine CPA showed preference for substrates with bulkier C-terminal AAs than MCCP, and only CPA had detectable activity against Cbz-Gly-Gly-Trp. These results are consistent with models that the AA substitutions in MCCP decrease the size of the active site pocket, conferring preferential and relatively narrower activity of MCCP against smaller C-terminal AAs. These studies show that MCCP shares the basic structure of pancreatic CPs at the level of both protein and mRNA but has divergent sequence and substrate specificity.

TUMOR NECROSIS FACTOR-ALPHA (TNF) SELECTIVELY DOWN-REGULATES A SUBPOPULATION OF HUMAN NEUTROPHIL C5a RECEPTORS (C5aR) AND PRIMES FOR A RESPIRATORY BURST STIMULATED BY C5a INTERACTING WITH THE RESIDUAL SUBPOPULATION OF C5aR. Robert D. Nelson, Department of Dermatology, University of Minnesota, Minneapolis, Minnesota.

Human neutrophils express an average of 200,000 C5aR's per cell and all receptors share a common affinity for C5a ($K_d = 10^{-9}$ M). We have determined that exposure of human neutrophils to 1 to 1,000 U/ml of TNF in vitro causes a dose-related decrease in the chemotactic sensitivity of treated cells to C5a and questioned the role of receptor down-regulation in this phenomenon. Using flow cytometry and fluorescein-C5a (FITC-C5a) to assay C5aR expression/function, we determined that TNF-mediated chemotactic desensitization of neutrophils to C5a apparently involved loss of receptors limited to approximately half of the C5aR's available. Scatchard analysis of ^{125}I-C5a binding before and after TNF treatment verified that this phenomenon reflected a reduction in receptor number rather than a decrease in receptor affinity. We then questioned the function of the "TNF-insensitive" subpopulation of C5aR's. Following pretreatment of neutrophils with 1,000 U/ml TNF, we monitored their respiratory response to rHuC5a in terms of H_2O_2 production. Control cells did not undergo a respiratory burst in response to rHuC5a at doses < 1 μM, but TNF-treated cells did undergo a respiratory burst to rHuC5a at doses of 10 and 100 nM. These findings demonstrate that C5aR's exist in two subpopulations based upon their sensitivity to down-regulation by TNF and that only the "TNF-sensitive" receptors mediate chemotaxis to C5a. They also demonstrate that the "TNF-insensitive" subpopulation of C5aR's is functional, but may require priming to become functional.

A PHYSIOLOGICAL DERMAL SUBSTRATE FOR WOUND HEALING. Gail K. Naughton, Leslie Jacob, and Brian K. Naughton. Marrow-Tech, Inc., La Jolla, California, and Hunter College School of Health Sciences, New York, New York.

A physiological skin substitute has been developed in our laboratory and is being studied in expanded preclinical trials. This substrate is a universal dermal equivalent consisting of human neonatal fibroblasts on a biodegradable mesh made of polyglycolic acid. The fibroblasts stretch across mesh openings, secrete collagens Type I and III, release growth factors, and remain mitotically and metabolically active. Sheets of dermal equivalent are sealed in Teflon bags and stored in liquid Nitrogen. Cells remain 90-95% viable after thawing. Preclinical trials to date have been performed on Charles River micropigs. Full-thickness wounds 4x4 cm in size were treated by application of dermal equivalent on mesh, mesh combined with growth factors, or mesh alone. A sheet of cultured autologous keratinocytes was applied to the dermal equivalent in vivo 10 days post dermal implantation. Areas treated by the universal dermis/autologous keratinocyte method showed rapid healing with minimal contraction and no evidence of rejection. Mesh was gradually hydrolyzed in vivo over 3-4 weeks. Wound areas which received mesh alone or a combination of mesh and growth factors showed substantial scarring. This mehtodology shows promise for providing a living wound treatment which can be stored frozen and used immediately in burn victims and patients with chronic decubitus ulcers.

MOLECULAR AND CELLULAR LOCALIZATION OF IL-8 AND IT'S INDUCER-TNF IN PSORIASIS. B. Nickoloff, J. Barker, C.E.M. Griffiths, J.T. Elder, S. Kunkel, V. Dixit. Depts of Pathology and Dermatology, Univ of Michigan.

One possible immunopathogenetic mechanism in psoriasis involves reciprocal interactions between activated T cells, macrophages/dermal dendrocytes (DD) in the dermis, with overlying hyperplastic keratinocytes (KCs). These important interactions are almost certainly mediated by increased local cytokine, and/or growth factor production by the resident and recruited cells themselves acting in an autocrine/paracrine fashion. Since cytokines including TNF-α coordinately regulate the temporal production of ICAM-1 and IL-8 by cultured KCs, we sought to study their expression in psoriasis.

Rabbit antisera to human IL-8 and TNF-α (both with a neutralizing titre of 1:25,000) were used with avidin-biotin immunoperoxidase staining of cryostat sections of either normal skin (N=4); or untreated active psoriatic plaques (N=4). Northern blot analysis was performed on normal (n=8) and psoriatic skin (n=8) by taking keratome biopsies, extracting total RNA and hybridizing with ^{32}P-labelled cDNA probes for IL-8, with cyclophilin serving as a reference control gene.

In normal skin, cyclophilin mRNA was present, but there was no detectable signal for IL-8 mRNA; IL-8 protein expression was confined to weak labeling of endothelial cells, and basal KCs with slight accentuation at the basement membrane zone (BMZ). TNF-α was only detected as weak expression on rare perivascular DD. In contrast, psoriatic plaques contained prominent staining of numerous DD for TNF-α. These positive dendritic cells were most prominent in "squirting papillae", filling the BMZ. Immediately above TNF-α containing DD, the KCs in the same area, expressed IL-8. IL-8 expression was confined primarily to basal KCs and corresponded to the same location of T cell epidermal infiltration and KC ICAM-1 expression. Both IL-8 and TNF-α reactivity by immunoperoxidase staining of the psoriatic lesions was abolished by pre-absorbing the anti-sera with highly purified IL-8 and TNF-α cytokines.

IL-8 mRNA was detected in 7 of 8 psoriatic biopsies as a strongly labelled single 2 Kb band.

These results clearly delineate important spatial cellular interactions between TNF-α containing DD with the overlying hyperplastic epidermal KCs, which have increased IL-8 and ICAM-1 expression. The ability of DD to produce TNF-α provides a means by which dermal cells can influence KCs in the initiation/recruitment (via IL-8) and retention (via ICAM-1) of T cell trafficking into the epidermis of psoriatic lesions. We propose that sequential and reciprocal KC-lymphohistiocytic cellular interactions evolve in the psoriatic lesional skin via appearance of immunomodulatory molecules such as TNF-α, IL-8, and ICAM-1 which are orientated in a temporally and spatially regulated fashion.

MID-INFRARED LASER ABLATION OF STRATUM CORNEUM ENHANCES TOPICAL DELIVERY OF DRUGS. J. Stuart Nelson, Jerry L. McCullough, Thomas C. Glenn, Beckman Laser Institute and Departments of Surgery (JSN) and Dermatology, University of California, Irvine, Irvine, California.

The stratum corneum (SC) provides the primary barrier for topical drug penetration. The removal of SC from pig skin by an erbium:YSGG laser (λ 2.79 μm) was assessed histologically and by electrical resistance measurements. The effects of laser treatment and tape stripping on the in vitro penetration of hydrocortisone (HC) and γ-interferon (γIF) were determined.

Excised pig skin was treated with laser (1 J/cm^2; 31 mJ/pulse; 1 Hz; 2 mm spot diameter). For skin penetration studies a total of 12 pulses was delivered to discrete 2 mm areas to ablate up to 50% of a total 3 cm^2 area. Franz in vitro skin penetration chambers were used to measure the cumulative 48 hr penetration of ^{3}H-HC and ^{125}I-γIF in laser treated and tape stripped skin.

Histological studies and electrical resistance measurements demonstrated that 10-14 laser pulses at the above energy density selectively ablated SC and abolished skin resistance. There was increased penetration of HC and γIF proportional to the area of ablation: 50% ablation produced 5x ↑ in HC; and 7x ↑ in γIF versus 1.3↑x with tape stripping.

These studies demonstrate that a mid-infrared laser can selectively and noninvasively destroy the skin barrier, facilitating penetration of large molecules such as γIF that can not penetrate intact skin. This new technique may be useful for both topical and transdermal delivery of therapeutic agents.

IDENTIFICATION OF ANTIGENS DEFINED BY BMZ ANTIBODIES THAT REACT TO BOTH THE EPIDERMAL AND DERMAL SIDE OF 1M NaCl SPLIT SKIN. Y Niimi, X-J Zhu, J-C Bystryn, Dept of Dermatology, NYU School of Medicine, New York, NY.

Some individuals have BMZ antibodies that react to both the epidermal and dermal side of skin split with 1M NaCl. The antigens defined by these antibodies and the significance of the finding is not known. To examine this problem we tested sera from 185 sequential pts with BMZ antibodies for reactivity to 1M NaCl split normal human skin. The antibodies in 173 (93.5%) pts stained only the epidermal side, in 6 (3.2%) pts only the dermal side, and in 6 (3.2%) pts both sides of the split skin. All sera with a combined staining pattern gave the same pattern when tested repeatedly with 3 different specimens of skin, indicating this pattern is reproducible and not an artifact of splitting the skin. By immunoblot analysis, 5 (83%) of the 6 combined staining sera reacted to a 160 kD antigen present only in epidermal extracts of normal skin, 1 reacted in addition to a 230 kD epidermal antigen, and 1 did not react to either epidermal or dermal extracts. In contrast, 5 (83%) of the 6 sera with dermal staining reacted to a 290 kD antigen present only in dermal extracts. Eighteen (90%) of 20 representative epidermal staining sera reacted to a 230 kD epidermal antigen and 7 (35%) sera (5 with the 230 kD antibody and 2 without) also reacted to the 160 kD epidermal antigen.

These results indicate that the combined staining pattern on 1M NaCl split skin is due to the presence of a distinctive BMZ antibody response directed predominantly to a 160 kD epidermal antigen and to an as yet unidentified dermal antigen.

MICROORGANISMS ASSOCIATED WITH PSORIASIS. Patricia W. Noah, E. William Rosenberg, Robert B. Skinner, Jr., and Vicki Baselski. Departments of Pathology and Medicine (Dermatology) University of Tennessee, Memphis, Tennessee, USA.

The microflora of 297 psoriasis patients was extensively examined. Throat, urine, and skin surfaces from scalp, ears, chest, face, axillary, submammary, umbilical, upper back, inguinal crease, gluteal-fold, peri-rectal, vaginal, pubis, penis, scrotal, leg, hands, feet, finger and toenail areas were cultured for aerobic bacteria, yeast, and dermatophytes. Antibody levels to streptococcal enzymes were performed (streptolysin-O, DNAse-B, hyaluronidase, STREPTOZYME). Giemsa smears and KOH preparations were also used to determine yeast and dermatophyte presence. Associated organisms thought to provoke a psoriatic attack were as follows: Streptococcal groups A, B, C, D, F, G, viridans, pneumoniae, Klebsiella pneumoniae, oxytoca, Escherica coli, Enterobacter cloacae, aerogenes, agglomerans, Proteus mirabilis, vulgaris, Citrobacter freundii, diversus, Morganella morganii, Pseudomonas aeruginosa, maltophilia, putida, Serratia marcescens, Acetinobacter calbio aceticus, luoffi, Flavobacterium specie, CDC groups Ve-1, Ve-2, EO-2, Bacillus subtilis, cereus, Staphylococcus aureus, Candida albicans, parapsilosis, Torulopsis, glabrata, Rhodotorula and dermatophytes. One or more anti-streptococcal enzyme tests was positive in 50% of patients. Titers to hepatitis E and to HIV were elevated in one patient each.

PURIFICATION OF PROLINE SPECIFIC ENDOPEPTIDASE FROM ORGANIZED GRANULOMAS. Y. Nozaki, T. Iida, Y. Sasaki, N. Sato and W.L. Epstein, Department of Dermatology, University of California, San Francisco, CA.

Proline specific endopeptidase (PSE; EC 3.4.21.26) is an endopeptidase which hydrolyzes mediators of inflammation such as bradykinin and angiotensin II. We measured PSE activity using Z-Gly-Pro-MCA as a substrate in experimentally produced granulomas (Gr) in skin and liver of C57BL/6 mice. PSE activity measured weekly during Gr development in skin for 5 wks showed an increase in activity corresponding to progress of the Gr tissue reaction, and was elevated up to 5.1 fold (7.2±0.8 mU/mg protein) over normal skin values. PSE activity in hepatic Gr also was elevated by 3.7 fold (8.4±0.9 mU/mg protein) as compared to normal liver. PSE then was purified from an extract of hepatic Gr in 20 mM Tris-HCl buffer, containing 10 mM each of EDTA and 2-mercaptoethanol, pH 7.0, by Q Sepharose Fast Flow column chromatography. The fraction with PSE activity which eluted with the extraction buffer in 0.1 M NaCl was further purified by gel filtration on a Sephacryl S-200, and finally on a HPLC TSK-250 column. PSE was purified 238 fold and molecular weight was estimated to be 79,000-77,000 by both HPLC gel filtration and SDS-PAGE electrophoresis. These findings suggest that PSE may be a modulator of Gr inflammation by metabolizing bioactive mediators.

FURTHER DEFINITION OF THE EFFECTS OF ULTRAVIOLET LIGHT ON ICAM-1 (CD54) EXPRESSION BY HUMAN KERATINOCYTES D.A. Norris, S.D. Bennion and M.H. Middleton Department of Dermatology, University of Colorado School of Medicine, Fitzsimmons Army Medical Center and the Denver Department of Veterans Affairs Hospital, Denver, CO

Induction of Intercellular Adhesion Molecule 1 (ICAM-1) on the surface of epidermal keratinocytes is crucial in initiating contact-dependent immunologic processes in the epidermis. Keratinocyte ICAM-1 can be induced by the cytokines gamma interferon and by tumor necrosis factor alpha (TNF-a). We have shown that ultraviolet radiation (UVR) initially inhibits and later strongly stimulates keratinocyte ICAM-1 induction.

We now report that sequential UVR exposure of human skin over three days induces immunoperoxidase-detectable ICAM-1 expression in epidermal keratinocytes in a dose-dependent fashion using radiation from a Sol-3 Solar Simulator (0,50 and 200 mJ/cm^2 UVB). In addition, by FACS analysis the induction of ICAM-1 on cultured human keratinocytes by low dose UVR (10 mJ/cm^2) is blocked by antibodies to TNF-a. The direct induction of ICAM-1 by TNF-a also can occur within 24 hours if low doses of TNF (.1 ng/ml) are used; higher doses of TNF-a are toxic to keratinocytes.

In summary, induction of ICAM-1 on human keratinocytes by UVR can be demonstrated both in vitro and in vivo, and this is due at least in part to the action of TNF-a, a potent inducer for ICAM-1 even at low concentrations.

A FUNCTIONAL TRANSFORMED ENDOTHELIAL CELL LINE IS TUMORIGENIC IN NUDE MICE. KA O'Connell*, G Landman*, ER Farmer*, MA Edidin+, Departments of Dermatology* and Biology+, The Johns Hopkins Medical Institutions, Baltimore, Maryland, USA.

We have recently described a stable SV40 transformed murine lymphoid endothelial cell line, SVEC4-10. Although SV40 is considered non-tumorigenic in mice, these studies were undertaken to assess the in vivo behavior of SVEC4-10 for the following reasons: (1) SVEC4-10 retains the functional in vitro characteristics of normal endothelial cells (FVIIIr:ag, high affinity LDL receptors, formation of tube-like structures in Matrigel, expression of class II MHC in response to gamma interferon, and binding of lymphocytes); and (2) published reports by others indicate a special relationship between endothelial cells and the middle T antigen of the related virus, polyoma, as reflected in hemangioma formation in middle T transgenic mice.

Intraperitoneal or subcutaneous injection of 10^6 viable SVEC4-10 cells into nude mice gave rise slowly to firm fixed nodules which progressed, causing widespread lethal disease. The tumors consisted of poorly differentiated mesenchymal cells invading connective tissues. No tumor cells were seen in parenchymal tissues. Staining of the tumor nodules revealed nuclear T antigen in the tumor cells. Tissue culture explants of the tumors consisted predominantly of host fibroblasts from which the transformed endothelial cells were eventually cloned. These findings show that SVEC4-10 is tumorigenic in nude mice and suggest a unique relationship between host fibroblasts and SVEC4-10 and/or between endothelial cells and SV40 T antigen.

AUGMENTATION OF GM-CSF EXPRESSION BY ULTRAVIOLET IRRADIATION IS MEDIATED BY IL-1 IN PAM 212 KERATINOCYTES. S. Nozaki[1], J. Abrams[2], M. Pearce[2] and D. Sauder[1], [1]Dept. of Medicine, McMaster University, Hamilton, Ont., CANADA and [2]DNAX Research Institute, Palo Alto, CA

Keratinocytes are a source of variety of cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF). Expression of keratinocyte cytokines can be modulated by ultraviolet light (UV). This study evaluates the modulation of GM-CSF in the murine Pam 212 cells by UV. Our results indicated that UVB irradiation (100, 200 J/m^2) augments GM-CSF mRNA expression (peaking at 32h). This increase in mRNA is associated with an increase in production of GM-CSF protein (peaking at 36-48h) from 13pg/ml in unirradiated cultures to 140pg/ml in UV irradiated cultures. In the same cell population, exposure to UVB increases interleukin 1 (IL-1) mRNA (peaking at 18h) and IL-1 protein as detected by bioassay. This increase in IL-1 precedes the increase of GM-CSF mRNA. Therefore, we sought to determine whether the UV-augmentation of GM-CSF mRNA expression was mediated by IL-1. Addition of recombinant IL-1 to the medium increases GM-CSF mRNA expression and leads to the subsequent increase in GM-CSF protein. Anti-IL-1 antibodies can completely inhibit UV-augmented GM-CSF mRNA expression. These results demonstrate that UVB augmentation of GM-CSF is mediated by IL-1 .

MOLECULAR ANALYSIS OF TYPE I (TYROSINASE NEGATIVE) OCULOCUTANEOUS ALBINISM W.S. Oetting and R.A. King, Dept. of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis, MN

Several types of oculocutaneous albinism (OCA) are thought to be linked to the gene coding for the melanin producing enzyme tyrosinase, including classical type I (tyrosinase-negative) OCA. We have begun to determine the location and type of the genetic lesion in the tyrosinase gene for several of our OCA families. DNA is isolated from peripheral white blood cells, and the polymerase chain reaction is used to separately amplify each of the five exons. The amplified product is then sequenced using di-deoxy sequencing with Taq polymerase. Family IAC89-1 contains a single child with type I OCA. The hairbulb tyrosinase activity (determined by the tritiated tyrosine assay) is absent in the proband and markedly reduced in both parents. Sequence analysis of this family shows a G to A substitution in the fourth exon (amino acid 401) that causes a glycine to arginine substitution in one allele of the proband and her family. This alteration is in the putative copper B binding site and we feel that this causes the lack of activity for the enzyme coded by this allele. We have also found a polymorphism at amino acid 174 that causes a serine to tyrosine substitution and which also abolishes a Mbo I restriction site. We have found this polymorphism in individuals who have normal tyrosinase activity levels and therefore conclude that this amino acid substitution does not affect tyrosinase activity. We are continuing these studies to determine the mutation in the second allele of this family as well as mutations in other families with tyrosinase-related albinism.

THREE IMMUNOLOGICALLY DEFINED POPULATIONS OF TRICHOHYALIN IN INNER ROOT SHEATH CELLS OF HUMAN HAIR FOLLICLES. W. Michael O'Guin, Motomu Manabe, and Tung-Tien Sun, Epithelial Biology Unit, Departments of Dermatology and Pharmacology, New York University School of Medicine, New York, New York.

Trichohyalin (TH) is a 220K protein found in trichohyalin granules (THG) of the human hair follicle. The function of this protein in inner root sheath (IRS) differentiation is not clearly understood, although it has been suggested that it serves as an intermediate filament associated protein or interfilamentous matrix molecule. In this study we have produced a panel of three distinct monoclonal antibodies (AE15-17) to human trichohyalin protein and have used them to trace the fate of TH during IRS differentiation. These studies have allowed us to define 3 immunologically distinct forms of trichohyalin. They are 1) the form of TH found throughout THGs and specifically recognized by AE15; 2) the form of TH which is localized as discrete punctae on THG and randomly distributed in the IRS cell cytoplasm--this epitope is recognized by AE16; and 3) the intermediate filament bound form of TH which is specifically associated with IRS filaments and is recognized by AE17. These studies have allowed us to determine unequivocably that TH is intermediate filament associated in vivo and that the function of TH is to laterally cross-link and precisely align parallel IRS filaments into bundles. Our results also indicate that TH binds to IRS filaments with specific binding sites with a 400 nm periodicity along the length of the filaments.

HUMAN NIDOGEN GENE: CHARACTERIZATION OF NUCLEOTIDE SEQUENCES AT THE 5'-FLANKING REGION AND EVIDENCE FOR FUNCTIONAL PROMOTER ACTIVITY. Joan O'Leary, Michael Fazio, Veli-Matti Kähäri, Yue Qiu Chen, Biagio Saitta and Jouni Uitto. Jefferson Med. College, Philadelphia, PA.

Nidogen is a sulfated multifunctional glycoprotein present in basement membranes. We have recently delineated the entire primary sequence of human nidogen through cDNA cloning (DNA 8:581-594, 1989). In this study, we have cloned the 5'-flanking region of the human nidogen gene. A ~35 kb clone (NCos4) was isolated from a human cosmid genomic library by screening with a 5' fragment of the nidogen cDNA. Hybridizations of EcoRI digested NCos4 with a 21 bp oligomer corresponding to the nidogen signal sequence allowed isolation of a 3.7 kb fragment. This subclone contained ~0.9 kb of 5'-flanking sequences of the gene. Nucleotide sequencing of the flanking DNA revealed the presence of a canonical CCAAT consensus sequence and a variant of the TATA motif, TATTT. One putative AP2 and several SP-1 binding sites were also present. To test the functional promoter activity of the 5'-flanking genomic DNA, several nidogen promoter/CAT reporter gene constructs were developed and analyzed in transient transfection of human and mouse cell cultures. Clearly detectable CAT activity was present in cells transfected with constructs spanning the promoter region from -1 to -885. Thus, the results suggest that the 5'-flanking region of the nidogen gene contains cis-acting regulatory elements necessary for transcription. The nidogen promoter/CAT gene constructs provide a means to study the transcriptional regulation of nidogen gene expression by trans-acting factors, and in human diseases of the basement membrane zone.

HYPERRESPONSIVENESS TO IL-4 AND HYPORESPONSIVENESS TO IL-2 OF PERIPHERAL BLOOD LYMPHOCYTES IN ATOPIC DERMATITIS. Mamitaro Ohtsuki, Masutaka Furue, and Yasumasa Ishibashi, Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Interleukin 4 (IL-4) has been shown to enormously enhance the production of IgE by B cells and also to induce the expression of IgE-Fc receptor on B cells, suggesting the possible involvement of IL-4 in the pathogenesis of atopic dermatitis (AD). IL-4 is also a potent T cell growth factor like interleukin 2 (IL-2). We examined IL-4 responsiveness and IL-2 responsiveness of peripheral blood lymphocytes (PBL) from 32 patients with AD and 20 non-AD controls by [^{3}H]-TdR incorporation assay of PBL. IL-4 responsiveness of the AD patients was found to be significantly higher than that of the non-AD controls, whereas IL-2 responsiveness of the AD patients was relatively lower than that of the non-AD controls. Therefore, the individual IL-4/IL-2 responsiveness ratio, the relative ratio of IL-4 responsiveness to IL-2 responsiveness, of the AD patients (52.16±15.61) was quite significantly higher than that of the non-AD controls (27.26±9.55, $p<0.001$). However, the correlation between the serum IgE level (logIgE) and the IL-4/IL-2 responsiveness ratio was not found (R=0.1198). The augmented IL-4/IL-2 responsiveness ratio found in the AD patients may possibly be related to the pathogenesis of AD.

TGF-ß2-INDUCED MODULATION OF HUMAN KERATINOCYTE GROWTH AND DIFFERENTIATION. David R. Olsen, Roberta W. Rhudy, Pedro Carrillo, and Karen Persichitte, Department of Cell Biology, Celtrix Laboratories, Collagen Corporation, Palo Alto, CA 94303.

The growth and differentiation of human keratinocytes is influenced by several growth factors including transforming growth factors α and ß. In this study we examined the effects of TGF-ß2 on ^{3}H-thymidine incorporation, the levels of ornithine decarboxylase (ODC), keratin 5 and involucrin mRNA in human epidermal keratinocyte (HEK) cultures as markers of growth and differentiation. ^{3}H-thymidine incorporation and ODC mRNA expression were down-regulated following exposure of HEKs to 1-50 ng/ml TGF-ß2. Inhibition of ODC expression was evident within 6 hours of exposure to TGF-ß2, continued exposure resulted in further decreases in ODC mRNA levels. Expression of TGF-α, a HEK mitogen, was also examined to determine if TGF-ß2 inhibited proliferation by turning off TGF-α production. TGF-ß2 treatment resulted in a slight increase in TGF-α mRNA levels. Inhibition of HEK growth following TGF-ß2 treatment was not accompanied by morphologic changes resembling those seen in cultures induced to undergo terminal differentiation, suggesting growth inhibition was not associated with differentiation. However, the same concentrations of TGF-ß2 which inhibited proliferation and ODC expression induced an increase in involucrin expression, a marker of keratinocyte differentiation. Expression of keratin 5 mRNA was unaltered by concentrations of TGF-ß2 as high as 50 ng/ml. These results suggest that TGF-ß2-induced arrest of keratinocyte proliferation, in itself, does not supply the appropriate signal to commit these cells to terminal differentiation even though specific markers of differentiation may be induced.

INDUCTION OF PROLIFERATING CELL NUCLEAR ANTIGEN/CYCLIN BY RETINOIC ACID IN SV40-TRANSFORMED HUMAN KERATINOCYTES. Natsuko Okada, *Sachiko Miyagawa, **Yoshinari Takasaki, and Kunihiko Yoshikawa, Department of Dermatology, Osaka Univ. School of Medicine, Osaka, *Department of Dermatology, Nara Medical Univ., Nara, and **Department of Internal Medicine, Juntendo Univ. School of Medicine, Tokyo, Japan.

The effect of retinoic acid on the expression of proliferating cell nuclear antigen (PCNA)/cyclin and its relationship with cell proliferation was studied in SV40-transformed human keratinocytes using an antibody from a lupus patient as the reagent. A good correlation between the effect of different concentrations of retinoic acid on PCNA/cyclin synthesis and cell proliferation was found. Immunofluorescence analysis of synchronously growing SV40-transformed keratinocytes revealed marked changes in the nuclear distribution of this antigen through the cell cycle, showing a striking increase at early S phase and a rapid decrease at the end of it. Addition of retinoic acid at a concentration of 10^{-5} or 10^{-6} M prolonged the duration of expression of this nuclear antigen when compared to non-treated cells. These changes were correlated with DNA synthesis as determined by autoradiogram. The cells cultured in basal medium for 24 hrs engaged in growth arrest and showed very little PCNA/cyclin, while the addition of 10^{-5} M retinoic acid resulted in an increase of PCNA/cyclin positive cells in 10 to 15% of total cells. These results indicate that the synthesis of PCNA/cyclin is correlated with DNA synthesis in SV40-transformed human keratinocytes, and that retinoic acid treatment can lead the cells into the S phase and induce the expression of this nuclear antigen.

INTERFERON ALFA-2a IN THE TREATMENT OF CUTANEOUS T-CELL LYMPHOMA **EA Olsen*, SJ Rosen**, and Roenigk HH****

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Forty-seven patients with a histological diagnosis of CTCL were treated with interferon alfa-2a (Hoffman-LaRoche, Nutley, NJ) over a 5½ year period. There were 26 males and 21 females with a mean age of 59 years. Stage of CTCL (according to the CTCL Cooperative Group) at study entry were as follows: T_1 = 12.8%, T_2 = 31.9%, T_3 = 21.3%, and T_4 = 34%. Patients were treated with 3 to 36 Mu of intramuscular interferon alfa-2a daily.

Overall, there was a 21.3% complete response (CR) rate, 31.9% partial response (PR) rate (≥ 50% regression cutaneous lesions) and 23.4% minor response rate (< 50% clearing cutaneous disease), for an overall 76.6% objective response rate. Two of 6 patients with T_1, 4/15 with T_2, 1/10 with T_3, and 3/16 with T_4 stage cutaneous disease had a CR. Taking into account extracutaneous disease, four of 11 patients with Stage I disease, 2/14 with Stage II, and 4/12 with Stage IV had a CR. The mean time to maximum response was 6.5 months.

Side effects were constitutional complaints in 100% of patients and weight loss/persistent GI distress in approximately 40% of patients. Most side effects were dose-related and all dissipated off treatment.

Interferon alfa-2a is an effective and well-tolerated treatment for all stages of CTCL.

EFFECT OF UV-IRRADIATION ON DNA DAMAGE AND REPLICATION OF CULTURED HUMAN MELANOCYTE. Hideo Onodera, Takashi Horikoshi, Hiroaki Eguchi, Kumiko Fukuzawa, Department of Dermatology, Sapporo Medical College, Sapporo, JAPAN.

The effects of UV-irradiation on DNA damage and replication were investigated using of human cultured melanocyte(MC). MC was grown in phenol-red free minimum essential medium supplemented with 20ng/ml of TPA(12-O-tetradecanoyl-phorbol-13-acetate) and 5% fetal bovine serum. At 90mJ/cm^2 of UVB irradiation, the maximum DNA synthesis was observed. At doses more than 90mJ/cm^2, DNA synthesis was inhibited. However, the unscheduled DNA synthesis was increased as the function of UVB doses. The replicative DNA synthesis was not detected at 720mJ/cm^2 of UVB irradiation. Under UVA irradiation, the maximum DNA synthesis was observed at 5J/cm^2 of UVA irradiation. The effect of UV-irradiation on DNA damage and replication of MC was more prominent in UVB than UVA.

The effects of UV-induced pigmentation were also investigated. The melanin content and tyrosinase activity were measured and compared 36 hours after the single exposure of UV in which the maximum DNA synthesis was induced. The tyrosinase activity was reduced by 50% under UVA and by 10% under UVB. The melanin content was also decreased soon after the UV-irradiation both in UVA and UVB by 10% and 40%, respectively. These data indicated that soon after the UV-irradiation, the melanogenesis of cultured MC is inhibited, however the transfer of melanin is rather promoted as seen MC in vivo.

Further studies must be needed to elucidate the mechanism of malignant transformation of MC using cultured MC.

EVALUATION OF SURFACTANT-INDUCED TOXICITY IN CULTURED HUMAN SKIN CELLS. Rosemarie Osborne, Mary A. Perkins, and Carol Hudson, Human & Environmental Safety Division, The Procter & Gamble Company, Cincinnati, Ohio.

Cultured human skin cells are a potentially useful model for preclinical screening of skin irritants. Human epidermal keratinocyte cultures (HuK) and keratinocyte-dermal fibroblast co-cultures (HuK/F) were treated with the skin irritant sodium dodecyl sulfate (SDS), a prototype anionic surfactant. SDS produced concentration-dependent decreases in cell viability, measured as incorporation of the vital dye neutral red (NR), and increases in release of: 1) lactate dehydrogenase (LDH), a measure of cytotoxicity; and 2) prostaglandin E_2 (PGE_2), a proinflammatory mediator. In HuK/F cultures treated 4 hrs with SDS, NR uptake decreased to 40% of control, LDH release increased 25-fold over control, and PGE_2 release increased 10-fold over control. Responses were maintained for up to 72 hrs. The half-maximal effective concentration was 40 to 70 μg SDS/ml for the 3 endpoints. SDS also stimulated PGE_2 release in HuK cultures (5-fold over control). NR uptake, and release of LDH and PGE_2 appear to be promising quantitative markers of surfactant-induced toxicity in cultured human skin cells.

PERCUTANEOUS ABSORPTION OF HYDROCORTISONE AND TESTOSTERONE AT THE VULVA AND FOREARM: EFFECT OF AGE AND SITE. Howard A. Oriba, Daniel A. W. Bucks, and Howard I. Maibach, Department of Dermatology, University of California, San Francisco, CA.

The percutaneous absorption of hydrocortisone (HC) and testosterone (TS) were studied following application to the vulvar and ventral forearm regions of pre- and post-menopausal women. Percutaneous absorption of HC was significantly greater at the vulva compared to the forearm in both pre- and post-menopausal women ($p < 0.01$, $p < 0.05$ respectively). Whereas, the percutaneous absorption of TS was significantly increased ($p < 0.01$) at the vulva of post-menopausal women only.

The effect of age on the percutaneous absorption of HC and TS was evaluated by using menopause as a biological chronometric end point. A commonly misconceived notion is that older skin has a diminished barrier and, therefore, percutaneous absorption would be greater. Our studies show that absorption of HC at the vulva of pre-menopausal was significantly greater ($p < 0.01$) than post-menopausal women. The ventral forearm of pre-menopausal women tended to have increased absorption compared to post-menopausal women, however, statistical significance was not achieved. No significant differences ($p > 0.05$) in the percutaneous absorption of TS at the vulva or forearm were observed between the two age groups.

SENSITIVE, SELECTIVE DETECTION OF CARCINOMAS AND MELANOMAS USING FLUORESCENT CATIONIC DYES. Allan R. Oseroff, Wendy Wan, Kalyan S. Wadwa, Kimberly A. Barrie, Young Park and James E. Whitaker. Department of Dermatology, Roswell Park Memorial Inst., Buffalo, NY; Spectroscopy Lab, MIT, Cambridge MA; Molecular Probes, Eugene OR.

Preferential accumulation of certain fluorescent cationic dyes by malignant cells should permit identification of these cells in situ. We investigated the potential of the cationic dye Nile blue A (NBA) and several new oxazine derivatives to detect human squamous (FaDu), breast (MCF-7), bladder (MB-49) and colon (CX-1) carcinomas and melanomas (CRL), grown subcutaneously in nude mice. This system provides a more rigorous test than distinguishing superficial or mucosal lesions, because of the shielding by overlying normal skin. Tumors ranged in size from non-palpable and essentially "invisible" (<1 mm), to >5 mm diameter, and were located 0.6-1 mm below the skin surface. Dyes were usually administered ip, but oral and topical preparations were also effective. Fluorescence lifetimes were measured with a picosecond laser, and intensities with a Laser Science Inc. nitrogen-pumped pulsed dye laser with a gated detector. No dye toxicity was observed. We found dye lifetimes <3 nsec in both tumors and skin. However, tumors had significantly greater fluorescence intensities, with ratios of [Fluorescence (tumor+overlying skin) /Fluorescence (normal skin)] ranging from 2:1 to > 5:1. Using NBA, the mean peak fluorescence ratio for squamous carcinomas was 2.6 $\pm$0.6 after 4.5 hrs ($p<.0001$, $n=31$). Bladder carcinomas and melanomas had mean ratios > 3.3:1. Detection specificity and sensitivity are much superior to that achieved with other non-cationic compounds including hematoporphyrin derivative (HPD). Fluorescent cationic dyes may be of value diagnostically for subcutaneous, superficial or mucosal tumors.

RETINOIC ACID AND OTHER INDUCERS OF TUMOR CELL DIFFERENTIATION INHIBIT THE INDUCTION OF PIGMENTATION IN CLOUDMAN MELANOMA CELLS. S. Orlow, A. Chakraborty, R. Boissy and J. Pawelek. Departments of Dermatology, Yale Univ. School of Med., New Haven, CT and Univ. of Cincinnati Coll. of Med., Cincinnati, OH.

Four compounds which induce many neoplastic cells to differentiate - retinoic acid (RA), hexamethylene bisacetamide (HMBA), sodium butyrate, and DMSO - all block the induction of pigmentation in Cloudman melanoma cells in response to melanocyte-stimulating hormone (MSH), isobutyl-methylxanthine or cholera toxin. The increase in tyrosinase which accompanies this induction was inhibited both in assays of intact cells and in extracts made from them. Activation of dopachrome isomerase, however, was inhibited only by RA. The effects of all four agents were independent of their effects on cell growth, and none of the agents directly inhibited tyrosinase, dopachrome isomerase, or tyrosine uptake. All of the agents stimulated the binding of MSH to intact cells. Examination of treated cells by electron microscopy revealed that both RA and HMBA blocked maturation of melanosomes at Stage II, and both inhibited the appearance of tyrosinase activity (detected by DOPA cytochemistry) in the trans-Golgi and coated vesicles. RA also caused the appearance of a "new" organelle, which appeared to represent an aberrant melanosome with disordered matrix filaments. The specificity of these four agents in terms of their effects on pigmentation identifies them as powerful tools which will be useful in dissecting the complex process of melanogenesis.

DNA SEQUENCE ANALYSIS OF THE HLA-DQβ1 LOCUS IN PATIENTS WITH DERMATITIS HERPETIFORMIS(DH). CC Otley, RJ Wenstrup, RP Hall, Dermatology, Duke Univ, Durham, NC.

Although 95 -100% of patients with DH express the HLA class II antigen DQw2, the role of HLA antigens in the pathogenesis of DH is unclear since 40% of normal subjects also express HLA-DQw2. HLA-DQ antigens which are serologically identical to those in normals but have a distinct primary structure by DNA sequence analysis have been associated with other autoimmune diseases. To determine whether a unique form of HLA-DQw2 is present in patients with DH, DNA sequence analysis of the highly polymorphic HLA-DQβ1 loci of 4 HLA-DQw2 positive DH patients was performed. The HLA-DQβ1 loci were amplified from genomic DNA by the polymerase chain reaction using oligonucleotide primers located within the HLA-DQβ1 exon. The amplified product was cloned into pUC18 and the DNA sequenced. The HLA-DQβ1 loci of 3 of 4 DH patients were identical to the previously described HLA-DQβ1 allele of normal HLA-DQw2 subjects. 1 DH patient and 1 HLA-DQw2 normal subject had a single base pair substitution resulting in the replacement of an arginine codon by a lysine codon in a putative antigen binding site at residue 70. These data suggest that patients with DH have a normal HLA-DQw2 β chain allele. The HLA-DQβ1 sequence containing the arginine to lysine codon substitution most likely represents a newly described normal allele of HLA-DQw2.

UV EFFECTS ON SKIN ANTIOXIDANTS. Lester Packer, Jurgen Fuchs, Jaana Leipala, Eric Witt, Ken Marenus, Daniel Maes, & Walter P. Smith Dept. of Mol. and Cell Biol., 251 LSA, Univ. of Cal., Berkeley, CA 94720, USA, and Estee Lauder Research and Development, Melville, N.Y.

A systematic study of the major water and lipid soluble antioxidant substances and enzymes affected by acute UV radiation (solar simulation), is underway. In excised skin of hairless mice, lipid soluble antioxidants, vitamin E and ubiquinones (UQ), are depleted partially by irradiation with UVA and more by irradiation with UVB. UVA causes some inactivation of catalase and small effects on the levels of reduced (GSH), oxidized (GSSG) and total glutathione, and slightly inhibits GSH reductase and peroxidase. UVB causes marked loss of total glutathione, increased ratio of GSSG to GSH, and inactivation of the glutathione enzymes. Vitamin C is also partially depleted by both UVA and UVB radiation, but superoxide dismutase is unaffected.

In vivo studies of the hairless mouse demonstrate similar effects on the loss of lipid soluble antioxidants due to skin UV light exposure. A range of single doses of UV light produced by a solar simulator was studied, from 0.25 J/cm^2 to 25 J/cm^2. At doses as low as 2.5 J/cm^2 a 10% loss of vitamin E and total quinols/ones was observed; at higher doses the loss of both antioxidants reached about 50%. Depletion of antioxidants stopped with the cessation of irradiation. Hence, skin antioxidant defense mechanisms are compromised by acute UV exposure, suggesting that a level of inactivation will be reached where molecular damage to lipid, protein, and DNA occurs.

TYROSINE TRANSPORT IN A HUMAN MELANOTIC MELANOMA CELL LINE
James M. Pankovich and Kowichi Jimbow. Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Canada.

While transport systems for tyrosine, i.e., the first precursor of melanin synthesis, have been studied in various cell types, little is known about its transport in melanoma cells. To develop a new anti-melanoma agent based upon melanin synthesis, this study characterized the transport mechanism of tyrosine in vitro using the human melanoma cell line SK-MEL23. Several tyrosine transport systems may be involved in pigment cells; systems L and T which transport neutral amino acids with branched or aromatic side chains and system ASC which transports neutral amino acids with smaller side chains. To determine which system or combination of these is involved in melanoma tyrosine transport, competitive inhibition studies were undertaken. The tyrosine transport was preferentially inhibited by the system L specific analog BCH (2-aminobicyclo-(2.2.-1)-heptane-2-carboxylic acid) and the system T substrate tryptophan. Sequential addition of these inhibitors at increasing concentrations indicated BCH and tryptophan to inhibit the same transporter. Our results suggest that tyrosine transport in the SK-MEL 23 cells is similar to system L transport characterized by BCH inhibition. This one transport system supplies all the tyrosine required for both cell growth and melanin synthesis. Thus, the stimulation of melanogenesis could increase the selective uptake of cytotoxic tyrosine analogs into the melanin synthetic pathway rather than general protein synthesis of melanoma cell, providing a basis for a tyrosinase mediated anti-melanoma effect.

GANGLIOSIDE G_{M3}, THE PREDOMINANT GANGLIOSIDE OF KERATINOCYTES, INHIBITS KERATINOCYTE PROLIFERATION. Amy S. Paller, Allison J. Ladner, and Eric G. Bremer, Departments of Pediatrics and Dermatology, Northwestern Univ. Medical School and Department of Immunology/Microbiology, Rush University, Chicago, IL.

Gangliosides have been implicated in regulating cell proliferation and differentiation, but their content and role in skin is unknown. Gangliosides were extracted from isolated human epidermis, undifferentiated cultured keratinocytes, and cutaneous squamous carcinoma cell lines, SCC 12 B2, SCC F2a and SCC 13, with chloroform:methanol. Lipids were partitioned by diisopropyl ether:1-butanol:aqueous sodium chloride, and the gangliosides were visualized by resorcinol treatment of thin layer chromatography plates. The total ganglioside concentration was 1.96 x 10^{-7} g sialic acid/mg dry weight epidermis from foreskin, leg or breast skin. The ganglioside content of undifferentiated keratinocytes from foreskin was 3.06 x 10^{-7} g sialic acid/mg dry weight. Ganglioside G_{M3} was the predominant ganglioside of isolated epidermis from foreskin, leg and breast skin (57.1%) and also of undifferentiated cultured keratinocytes (76.3%). From isolated epidermis, G_{M2} (16.7%) and G_{D3} (15.8%) were other major ganglio- sides. Polysialogangliosides were found in small amounts. G_{M1} was only detectable in trace amounts in 2 of the 3 samples of breast skin and was undetectable in foreskin or leg skin. In cultured keratinocytes, the types and relative percentages of gangliosides were similar to those of intact epidermis, and G_{M1} was undetectable by densitometry or by immunostaining with anti-G_{M1} antibody or HRP-conjugated cholera toxin. The SCC lines cultured in low calcium keratinocyte growth medium had largely G_{M3} and a significant amount of G_{M2}, as did the normal undifferentiated keratinocytes, but the amount of membrane G_{D3} was markedly reduced (0.7%) and G_{M1} was clearly detectable (2.3%). Purified (NeuAc)G_{M3}, added to undifferentiated cultured keratinocytes, inhibited proliferation of cells without toxicity in a dose-dependent manner at concentrations of 25-100 μg/ml, but concentrations of (NeuAc)G_{M3} in the same range were toxic to all of the SCC lines. Ganglioside G_{M1} did not show a similar inhibitory effect. These data suggest that ganglioside G_{M3} may participate in the regulation of normal and neoplastic keratinocyte proliferation in vitro and perhaps in vivo.

SKIN CAPILLARIES SURROUNDING CHRONIC VENOUS ULCERS DEMONSTRATE SMOOTH MUSCLE CELL HYPERPLASIA AND INCREASED LAMININ AND TYPE IV COLLAGEN. Jeffrey B. Pardes, Marcia G. Tonnesen, Vincent Falanga, William H. Eaglstein, and Richard A. F. Clark, Depts of Medicine, Pediatrics and Dermatology, SUNY, Stony Brook, NY; National Jewish Center, Denver, CO; and Univ. of Miami, Miami, FL.

Chronic ulcers often form on the lower legs of patients with venous insufficiency. Although cutaneous hypoxia and venous hypertension are predisposing factors, the pathophysiology of these ulcers is unclear. Previous studies have reported that dermal capillaries are increased in number, have increased "vascular endothelial volume," and are "cuffed" with fibrin deposits. One theory proposes that the fibrin "cuffs" may block oxygen diffusion. We have reexamined the dermal vessels surrounding venous ulcers to better define the capillary pathology. Small wedge biopsies from the margin of venous ulcers in 7 patients were fixed in acid alcohol and embedded in paraffin. Adjacent sections of these specimens were stained for fibrin/fibrinogen, fibronectin, laminin, type IV collagen, muscle-type actin (a smooth muscle cell marker in the skin), and von Willebrand factor (an endothelial cell marker) with FITC-labeled avidin-biotin complexed antibodies. We observed that fibrin deposits occurred around many but not all capillaries, and often failed to encompass vessels completely. Capillary walls were markedly thickened with increased cellularity and increased extracellular matrix (ECM) material. The increased cellularity was attributable to smooth muscle cell (? pericyte), not endothelial cell, hyperplasia. The increased ECM consisted of laminin and type IV collagen but usually not fibrin nor fibronectin. Capillaries surrounding normal acute cutaneous wounds do not manifest these alterations. We speculate that the capillary smooth muscle cell hyperplasia and accompanying increased ECM may contribute to venous ulcer disease, perhaps by creating a diffusion barrier to oxygen and nutrients and by constricting the capillary bed, thus reducing local blood flow.

INTERACTIONS OF HARD AND SOFT KERATINS IN TRANSFECTED EPITHELIAL CELLS. Susana Pang, Da-Wen Yu, Daniel M. Checkla, Tung-Tien Sun, and Arthur P. Bertolino, Epithelial Biology Unit, Departments of Dermatology and Pharmacology, New York University Medical Center, New York, New York.

On the basis of sequence homology data, keratins were divided into Type I (acidic) and Type II (neutral-basic) classes. More recently, Conway and Parry (Int. J. Biol. Macromol., 1988, 10:79-89) compared the homology scores for the rod domains of "hard" keratins of the epidermal appendages (hair, nails) and "soft" keratins of epithelia. These additional data support further subdivisions into Type Ia and Type IIa ("hard" keratins) and Type Ib and Type IIb ("soft" keratins) subclasses. They proposed that "hard" and "soft" keratins may not be able to copolymerize, given the significant sequence divergences. To test this hypothesis, we transfected HeLa cells, which express endogenous "soft" keratins, with expression vectors containing cDNAs which encode a mouse Type Ia hair keratin or a mouse Type IIa hair keratin. Using a panel of antibodies with different specificities, we have shown that both "hard" keratins can integrate into the endogenous "soft" keratin network of the HeLa cells. In some cells, the "hard" keratin formed aggregates, presumably reflecting an overproduction of the exogenous protein. These results suggest that "hard" and "soft" keratins can copolymerize despite their sequence divergences.

HUMAN LAMININ A CHAIN: IDENTIFICATION OF STRUCTURAL FEATURES DEDUCED FROM cDNA SEQUENCE ANALYSES. M. Gabriela Parente, Michael J. Fazio, Rupert Timpl, Mon-Li Chu and Jouni Uitto. Jefferson Medical College, Philadelphia, PA.

We have recently isolated laminin A chain cDNA clones by screening a human placental λgtl1 library with a mouse laminin A chain cDNA probe (Lab. Invest. 60:772-782, 1989). The largest clone (λpA-1) which consisted of 2,232 nucleotides corresponded to the C-terminus of the laminin A chain. In this study, a 5' 0.7 kb fragment of λpA-1 was used to screen a human skin fibroblast λZAP cDNA library. Screening of ~5 x 10^5 plaques identified one positive recombinant phage, λpA-3. Restriction mapping revealed that λpA-3 was 1.4 kb and extended ~0.9 kb upstream from λpA-1. Nucleotide sequence analyses of clones λpA-1 and λpA-3 revealed a total of 3,042 nucleotides, with an open reading frame encoding 950 amino acids. There was an unusually low nucleotide sequence identity, ~74%, between human and mouse laminin A chain in this region, which encodes a unique globular domain not present in laminin B chains. The deduced amino acid sequences revealed the presence of internal repeats, ~150 residues each, a characteristic feature being the repeating sequence YXGGLP. Twelve cysteines were present in human sequences, and their location, with a single exception, was invariant as compared with mouse sequences. Interestingly, the human sequence revealed the presence of an RGD motif, a putative cell recognition site. Thus, the amino acid sequences deduced from human cDNAs identified interspecies conserved features which may have biological importance in laminin interactions with cells and other extracellular macromolecules.

IMAGE ANALYSIS OF METASTASIZING CLINICAL STAGE I MELANOMA IN THICKNESS RANGE 1.0 - 2.5 MM; TIME TO METASTASIS AND SURVIVAL ARE RELATED TO LYMPHOCYTIC INFILTRATES. G.C. Pastorfide, H.R. Byers, R.L. Barnhill, A.J. Sober, A.L. de Roa, and M.C. Mihm Jr. Dermatopathology Division, Department of Pathology and Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts.

A retrospective pilot study with fifteen year follow-up identified eleven patients out of 194 who were clinical stage I melanoma in the thickness range 1.0mm - 2.5mm and who developed metastases. These patients' primary tumors were analyzed using routine microscopy and image analysis without knowledge of outcome. Increased lymphocytic infiltrates within the tumor (tumor infiltrating lymphocytes; TIL) and beneath the base of the tumor in the surrounding tissue (non-TIL) significantly correlated with delayed time to metastasis (p=0.014 and p=0.000, respectively) and a longer survival period (p=0.045 and p=0.001, respectively). Other tumor parameters such as thickness, width, cross-sectional area, level, and number of mitoses did not correlate with time to metastasis and survival. Image analysis thus provided an objective quantification of tumor parameters and lymphocyte response, with both TIL and non-TIL being the most important parameters in this thickness range of melanoma in patients who developed metastases. Lymphocytic infiltrate area at the tumor base in relation to tumor area was of prognostic value: the larger the ratio, the greater the time interval from metastasis to death (p=0.008). The correlation of increased quantities of lymphocytes with delayed metastasis supports a biological role of the lymphocytic infiltrate in this category of patients.

DOPACHROME CONVERSION FACTOR FUNCTIONS AS AN ISOMERASE. John M. Pawelek, Ashok Chakraborty, Marilyn Murray, and Seth Orlow, Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Dopachrome conversion factor is an enzymatic activity associated with the pigmentary system which catalyzes the conversion of dopachrome, an intermediate in melanin biosynthesis, to dihydroxyindole-2-carboxylic acid (DHICA). To date, the mechanism of action of DCF has been unknown because all previous assays have employed a dopachrome substrate contaminated with L-dopa. It has therefore not been possible to determine whether L-dopa acts as a hydrogen donor in the reaction. In this study it is shown that DCF, purified more than 200-fold, catalyzes the conversion of dopachrome to DHICA equally well in the presence or absence of L-dopa. Since dopachrome and DHICA are isomers of the elemental composition $C_9H_7NO_4$, the DCF-mediated reaction thus appears to be an isomeric rearrangement of hydrogen ions from one portion of the dopachrome molecule to another. The isomerization apparently involves an intramolecular oxidoreduction which results in a tautomeric shift. Such reactions are catalyzed by isomerases. The results indicate that the name "dopachrome isomerase" appropriately describes the function of DCF.

THE ROLE OF REACTIVE OXYGEN ($^1O_2$, $O_2^{\cdot-}$, $^{\cdot}OH$) IN COLLAGEN CROSS-LINKING AND PHOTOAGING. M.A. Pathak, M. Daile Carbonare, S. Mobilio, Department of Dermatology, Harvard Medical School, Boston, MA.

Free-radicals and reactive O_2 species ($^1O_2$, $O_2^{\cdot-}$, $^{\cdot}OH$) are generated by UVB (290-320nm), UVA (320-400nm) or by skin photosensitization reactions involving riboflavin (RF), porphyrins (HPD) and psoralens. Our invitro and invivo studies indicate singlet O_2 ($^1O_2$) may be involved in collagen denaturation and photo-induced cross-links. Solutions of collagen (2 mg/ml) extracted from guinea pig skin in acetic acid (0.1 - 0.5M) were irradiated with varying doses of UVB (30-600 mj/cm^2), UVA (1-20 J/cm^2) with or without RF, HPD, or 3-carbethoxy-psoralen (3-CP) (10^{-4}-10^{-6}M). The production of $^1O_2$ was measured by recording optical density changes at 440nm of N,N-dimethyl p-nitro-soaniline. The production of $O_2^{\cdot-}$ was measured at 560nm using nitroblue tetrazolium. The irradiated collagen, before and after dialysis, revealed gel-like precipitates which increased in the presence of O_2 or D_2O. Collagen precipitation decreased significantly under anoxic conditions. Both UVB and UVA, with and without RF, HPD, or 3-CP generated increasing amounts of $^1O_2$ with increasing UV dose. Concomitantly, the soluble form (non-cross linked) of collagen decreased and insoluble (cross-linked) collagen increased. SDS gel electrophoresis of irradiated collagen revealed increased values of cross-linked protein. The induction of protein cross-links was inhibited when collagen was irradiated in the presence of $^1O_2$ quenchers (NaN_3, ß-carotene, α-tocopherol acetate) but not with t.retinoids. Singlet oxygen appears to play a major role in photoaging of skin.

A 70K PROTEIN CHARACTERIZES THE ECTODERMAL DYSPLASIA, PACHYONYCHIA CONGENITA. Johann Peikert, Steven Sundby, *Siti Aisah Boediardja, Maria Hordinsky, Dept. of Dermatology, University of MN, Mpls. MN, USA, and *Dept. of Dermatology, University of Indonesia, Jakarta, Indonesia.

Previous work has suggested that a unique, non-keratin 70K protein found in nail clippings characterizes pachyonychia congenita (PC). In this study, we asked if this 70K protein is found in others with PC, and examined which part of the nail complex contains this protein. We examined two families with PC, one with autosomal dominant (AD), type II PC and the other with type III PC, of either autosomal recessive transmission or spontaneous mutation for the AD form. The first family was Caucasian, and consisted of an affected father (Patient 1) and son (Patient 2), and an unaffected mother. A second Indonesian family included 2 unaffected parents and 7 siblings as well as an affected son (Patient 3). Samples of hair and distal finger- and toenail clippings were collected from all subjects. Subungual hyperkeratotic material (SUHM) from patient 1 and plantar skin from patients 1 and 3 were also collected. All samples were solubilized by incubating with 8 M urea, 20 mM dithioerythritol, and 50 mM Tris-base, pH 8.0. Extracted proteins were alkylated, lyophilized and analyzed with SDS-PAGE. A prominent 70K protein and a less prominent 80K protein were found in distal finger- and toenail clippings and SUHM from affected patients. A 70K protein was present in smaller amounts in affected plantar skin, fingernails from patient 3's father and toenails from patient 3's father, mother and one sibling. Both proteins were absent in all hair samples. Whether the 70K and 80K proteins are either structurally or functionally associated remains to be determined. The presence of the 70K protein in all affected members of 2 distinct families and certain clinically unaffected family members of patient 3, suggests its possible use as a biochemical marker for PC. Because the hyponychium is very similar to plantar skin, the presence of the 70K band in SUHM and hyperkeratotic plantar skin supports the hypothesis of hyponychium as the source of subungual hyperkeratosis in PC.

HISTOLOGICAL DEMONSTRATION OF MACROPHAGE SUB-POPULATIONS IN WOUND GRANULATION TISSUE. Kevin F M Patrick, Jerry J Pickworth, ConvaTec Wound Healing Research Institute.

We have used two stains to identify macrophages in wound tissue - chromotrope 2R (C2R) staining lysosomal granules, and peanut agglutinin (PNA), with an immunoperoxidase label, staining macrophage cell surfaces. Initial results showed that approximately 50% of cells stained with C2R were identified by the PNA method in wound tissue excised 17 days post operatively, indicating possibly two distinct populations of wound macrophages, the study has been extended to include days six, nine and twelve days post operatively. Wounds treated with dry dressing (Johnson & Johnson NA) Polyurethane Film (Opsite, Smith & Nephew) Hydrocolloid dressing (Duoderm, Squibb ConvaTec) have been examined. Currently a double staining silver-enhanced immunogold method is being employed to determine whether two or three sub-populations exist, and to try to assess specific macrophage activity in the healing wound.

FASCIITIS WITH EOSINOPHILIA ASSOCIATED WITH L-TRYPTOPHAN INGESTION: EVIDENCE FOR ACTIVATION OF COLLAGEN GENE EXPRESSION. Juha Peltonen, Stephan Sollberg, Li Li Hsiao, John Varga, Sergio Jimenez and Jouni Uitto, Departments of Dermatology, Medicine, and Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia PA.

Five patients with rapidly progressing induration of the skin and peripheral eosinophilia (upto 50%), and with a history of L-tryptophan intake (upto 2.6 g/day) for 2-5 months prior to onset of the disease were studied. Histology of the affected skin demonstrated markedly thickened fascia with accumulation of collagen. There were dense infiltrates of eosinophils within the fascia, and numerous eosinophils were also distributed throughout the dermis. Skin biopsy specimens were examined for evidence of activation of collagen gene expression by in situ hybridizations with radioactively labeled cDNAs specific for human α1(I) or α2(VI) collagen sequences. Autoradiographic detection of [^{32}P]cDNA-mRNA hybrids revealed that a subpopulation of cells in fascia and deep dermis actively expressed type I and VI collagen genes. In contrast, only a few cells showed positive hybridization signal in the mid and upper dermis of the same sections. Thus, the presence of eosinophils and the activation of collagen gene expression by fibroblasts demonstrated a discordant spatial distribution. The results suggest that L-tryptophan, or one of its metabolites, activates collagen gene expression in the deep dermis and fascia, and such activation may not directly involve eosinophils.

UVB-INDUCED PROSTAGLANDIN SYNTHESIS IS STIMULATED BY ENDOGENOUS HISTAMINE IN HUMAN SKIN EXPLANTS. Alice P. Pentland, and Susan C. Jacobs, Div. of Dermatology, Washington Univ., St. Louis Mo. 63110.

Early UVB erythema is mediated by prostaglandin (PG) synthesis. Mast cells release the PG agonist histamine 4-6 hours after UVB exposure, suggesting histamine may stimulate UVB-induced PG release. Human skin explants were used to study if UVB-induced endogenous histamine release modulates explant PG synthesis. Skin was defatted and cut into 0.1 gm squares. Explants were incubated in serum-free medium at 37°C overnight, then exposed to between 30 and 180 mj/cm^2 UV using Westinghouse FS20 lamps. At intervals after UV, PG in the medium was assayed by RIA. A 4-fold increase in PGE_2 released was found in explants exposed to $\geq$ 60 mj/cm^2 UVB. Enhanced release of PGI_2 (measured as 6-keto $PGF_1\alpha$) and $PGF_2\alpha$ was also found. Incubation of the explants with 50 µM brompheniramine (an H1 blocker) but not cimetidine (an H2 blocker) decreased irradiated cultures PGE_2 release 60%. Drug addition did not affect PG production of controls. Sups from irradiated tissue contained only 25% more histamine than controls as measured by RIA, suggesting UV increased agonist responsiveness. Explants were therefore stimulated with 1-10 µM histamine or .01-10 µM bradykinin. Control explant PG synthesis increased minimally in response to the agonists, while 3 µM histamine and 100 nM bradykinin increased irradiated explant PG synthesis 3x. These observations suggest that 1) UVB irradiation changes the threshold and magnitude of agonist-induced PG synthesis in human skin and 2) endogenous histamine stimulates UVB-induced PG synthesis in humans.

CREMOPHOR-INDUCED INCREASE IN HUMAN SKIN PERMEABILITY LK Pershing and J Corlett, Dept. Medicine (Dermatol), Univ. of Utah, Salt Lake City, UT.

Oral administration (10 mg/kg/d) of cyclosporine A (CSA; Sandimmune^{tm}; Sandoz) increases in vivo human skin permeability to topically applied compounds via an increase in skin diffusivity (Clin Res 37:766A, 1989). Subcutaneous (SC) admininstration of CSA in vivo decreases diffusivity thereby decreasing skin permeability. Similar concentrations of CSA are present in the stratum corneum of the human skin sandwich flap (HSSF) however, following 1,3 and 7 days with either route of administration. Sandimmune^{tm} vehicle contains cremophor EL (CR)which produces side effects in other organ systems and which might effect skin permeability. In vivo experiments were designed therefore, to investigate the influence of oral or SC administration of CR on in vivo human skin permeability to topical 14C caffeine in ethanol (SA= 47.5 mCi/mMol; NEN) using the HSSF. Oral or SC administration of CR-alone increased human skin permeability to caffeine 4 X above that with oral CSA-plus-CR. Topical administration also increases human skin permeability to caffeine. Increasing the duration of oral, subcutaneous or topical CR pretreatment results in a progressive increase in human skin permeability in vivo. These changes in skin permeability occur without a significant alteration in transepidermal water loss or capillary permeability as determined with an evaporimeter and surface dermofluorometer, respectively. These data demonstrate that CR in Sandimmune^{tm} formulation is responsible for the increased permeability observed with the administration of Sandimmune^{tm}.

MAST CELLS, NEUTROPHILS AND EOSINOPHILS IN PRURIGO NODULARIS. Gregory L. Perez, Margot S. Peters, Ashraf M. Reda, Joseph H. Butterfield and Kristin M. Leiferman, Departments of Dermatology and Medicine, Mayo Clinic, Rochester, MN.

Prurigo nodularis is a disease of unknown etiology. In order to characterize the role of mast cells, neutrophils and eosinophils, we analyzed seven biopsy specimens by indirect immunofluorescence for mast cell tryptase, for neutrophil elastase and for eosinophil granule major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). Mast cells were detected in all of the specimens with prominent numbers of mast cells in four specimens; there was minimal or no extracellular deposition of tryptase in any of the tissues. Neutrophil infiltration was observed in all specimens but few cells were observed in four; extracellular elastase was minimal or absent in all but one specimen in which prominent dermal elastase deposition was found. Scanty eosinophil infiltration was present in all specimens; however, extracellular deposition of the eosinophil granule proteins including MBP, EDN and ECP was present in all but one specimen and striking deposition of at least one eosinophil granule protein was present in five of the seven specimens. These studies suggest that mast cell numbers may be increased in prurigo nodularis but that mast cell degranulation and neutrophil degranulation are not associated with persistent lesions of prurigo nodularis. In contrast, eosinophil degranulation as evidenced by striking deposition of granule proteins is prominent in persistent lesions. Each of the eosinophil granule proteins studied have potent effects on tissues; the toxicity of these proteins and their deposition in lesional tissue suggests a pathogenic role of the eosinophil in prurigo nodularis.

ESTABLISHMENT AND CHARACTERIZATION OF A CELL LINE DERIVED FROM ULTRAVIOLET INDUCED MALIGNANT MESENCHYMAL TUMORS IN HAIRLESS ALBINO MICE. RG Phelps, DS Kohtz, RE Gordon, E Schwartz, Dept. of Dermatology, Mount Sinai School of Medicine, New York, N.Y.

Skh/hr-1 hairless albino mice were subjected to an intense incremental ultraviolet irradiation schedule to produce tumors which have the histologic features of malignant fibrohistiocytic neoplasms. Electron microscopy, and immunochemistry showed both fibrocytic and histiocytic differentiation. The tumors were finely minced with sterile technique in Hank's media and inoculated en masse subcutaneously in syngeneic mice. After several weeks multiple dermal nodules developed which had histologic features similar to the primary tumor, though these secondary tumors showed limited potential for further growth. Parallel explants were plated initially in 20% fetal calf serum (FCS) and 1% bovine embryo extract and subsequently in 10% FCS in Dulbecco's modified Eagle's medium and within several weeks a cell line with distinctive features emerged. There were confluent, overlapping masses of non-contact inhibited cells which were filiform, fusiform and bipolar, admixed with flattened almost "squamoid" appearing cells. This morphology was maintained in perpetuity despite repeated passaging. Electron microscopy showed that these cells formed as many as three layers, had prominent filopodia, lysosomal structures, endoplasmic reticulum, and no intercellular junctions-suggesting histiocytic differentiation. Isolated cells were cloned from this polyclonal parent culture and the clones showed a similar colonial morphology: bipolar cells and admixed flattened cells. The polymorphism of this cell line and tumor, its reproduction in vivo and in vitro support the concept that fibrohistiocytic tumors are derived from one cell type capable of multiple lines of differentiation. Further studies on this line may help understand mechanisms of dermal photocarcinogenesis and the presumed histogenesis of dermal mesenchymal tumors.

INHIBITION OF RESPONSE TO ALLOANTIGEN BY INFUSIONS WITH PHOTOINACTIVATED CD4+ T CELL CLONES. M. Perez, L. John, Y. Yamane, C. Janeway and R. Edelson. Depts. Dermatol. and Immunol. Yale Univ., New Haven, Ct.

Induction of tolerance to skin allotransplantation by infusions of photoinactivated alloreactive lymphocytes has been demomstrated. This tolerance has been adoptively transferred to syngeneic recipients by Thy1+, Lyt2+, L3T4- lymphocytes. In order to identify the cells inducing such an immunosuppressive response to alloantigen, D10 clones (CD4+,CD8- cloned murine T cell line specific for conalbumin (CA) associated with I-A^k and alloreactive to H-2^b) were photoinactivated with 8-methoxypsoralen (8-MOP) and ultraviolet A light (UVA) prior to intravenous (iv) infusion into AKR/J mice which then were challenge with H-2^b lymphocytes in a Delayed Type Hypersensitivity (DTH) response.

D10 clones were fed every three weeks with H-2^k mitomycin C inactivated feeder cells and 100ug/ml of CA in Clicks media containing 5%T-cell Growth Factor and 5% Fetal Calf Serum. Ten days post stimulation, D10 clones were incubated in 100ng/ml of 8-MOP and exposed to 1Joule/cm^2 of UVA light, aliquoted at 2.5x10^6 cells/mouse in 200ul of PBS and iv injected into the tail vein of ten AKR/J mice. AKR/J mice received iv infusions of 8-MOP/UVA treated D10 clones biweekly x six. Control mice received similar amounts of viable D10 clones. All mice were primed in the flank and footpad challenged with H-2^b cells, measuring footpad swelling after 24 hrs.

AKR/J mice recipient of 8-MOP/UVA treated D10 clones were 56% suppressed in DTH response to H-2^b alloantigen as compared to controls. Therefore, tolerance to skin allotransplantation is induced by phenotypically helper T cells.

SYNTHESIS OF PROTEOGLYCANS AND GLYCOSAMINOGLYCAN FREE CHAINS BY CULTURED HUMAN ICHTHYOTIC KERATINOCYTES. MW Piepkorn, P Fleckman, H Carney, A Linker, Depts of Medicine and Pathology, Univ of Utah and the VA Hospital, Salt Lake City, UT, and the Dept of Medicine, Univ of Washington, Seattle, WA.

Ichthyosis vulgaris is a genetic dermatosis in which retention of partially-differentiated keratinocytes is thought to create clinical hyperkeratosis. Since proteoglycans are components of keratinocyte membranes, we hypothesized that alterations in their synthesis could explain the retention hyperkeratosis. Keratinocyte lines were established from blistered epidermis from 2 subjects of a kindred with ichthyosis vulgaris and were analyzed in parallel with keratinocytes from an age and sex matched control. Duplicate confluent cultures of each line were steady-state labeled with [35S]-sulfate and [3H]-glucosamine. The medium and detergent extracts of the cell layers were coded and analyzed by standard methodology for proteoglycan content, without knowledge of source. Within the medium of all 3 lines, the principal labeling was in proteoglycans of relatively low molecular mass (Kav 0.54 by Sepharose CL-4B, ~50 kDa), of which ~20% was heparan sulfate and ~80% chondroitin sulfate. The proteoglycan nature of the products was confirmed by sensitivity to alkaline borohydride reduction. Detergent extracts of the cells contained a mixture of proteoglycans and apparent ~12 kDa glycosaminoglycan free chains (i.e., resistant to alkaline reduction), in a ratio of ~1:4. Heparan sulfate and chondroitin sulfate classes were roughly equally represented among both the proteoglycans and the free chains from the cells. Total amounts of product normalized to the DNA content of the cultures were similar for all 3 cell lines, and qualitatively the labeling patterns between each were indistinguishable. These data suggest that proteoglycan and glycosaminoglycan free chain synthesis in ichthyotic keratinocytes is unchanged from that of normal cells.

MEMBRANE LIPID ALTERATIONS *IN VIVO* FOLLOWING 8-MOP ADMINISTRATION FOR EXTRACORPOREAL PHOTOCHEMOTHERAPY. Piette WW, Walker KE, Strauss RG, Koerner TAW. Departments of Dermatology and Pathology, University of Iowa College of Medicine, Iowa City, Iowa.

Extracorporeal photochemotherapy (ECP) may act through an immune response to an altered subpopulation of lymphocytes. Current understanding of immunology suggests that a response to such lymphocytes is more likely to result from alterations of their cell membrane than of their nuclear DNA. While UVA-activated 8-methoxypsoralen (8-MOP) binds to DNA, 8-MOP structure suggests binding to lipid might also occur. To test this hypothesis *in vivo*, paired samples (n=8) of membrane lipid fractions from mononuclear blood cells (Ficoll-Hypaque separation) were obtained from ECP-treated patients with cutaneous T-cell lymphoma (n=5) before and after 8-MOP administration. Quantitative comparison in mole-percent (x ± SEM) of lipid components by high performance thin layer chromatography ($CHCl_3$-CH_3OH-H_2O, 65:25:4) showed a band at Rf 0.42 that was 4.1 ± 0.6 in the pre-8-MOP group and 2.7 ± 0.7 in the treated group. The reduction in this band was significant ($p < 0.05$). This band is distinct from the those of the major phospholipid and neutral lipid membrane components. These data suggest that UVA-8-MOP interaction with membrane lipids occurs, and offer a mechanism for 8-MOP-induced cell membrane alterations. The possible effects of such changes on efficacy or toxicity of treatment will require further study.

INCREASED VASOACTIVE INTESTINAL POLYPEPTIDE LEVELS IN LESIONAL SKIN OF PSORIASIS. C.Pincelli, F.Fantini, P.Romualdi*, G.Lesa*, A.Giannetti, Department of Dermatology, University of Modena and *Institute of Pharmacology, University of Bologna, Italy.

Psoriasis is known to have exacerbations and remissions affected, among other factor, by emotional stress. This suggests a possible role for neuropeptides in the pathogenesis of psoriasis. Moreover, psoriasis is characterized histologically by inflammation and dilated capillaries in the papillary dermis. Vasoactive intestinal polypeptide (VIP) is a widely distributed 28 aminoacid peptide which acts as a neurotransmitter mostly involved in vasodilation and glandular secretion. Immunohistochemical studies have shown VIP - immunoreactive (IR) nerves in the skin mainly around blood vessels and sweat glands. The aim of the present study was to evaluate the levels of VIP-IR in skin from psoriatic patients by radioimmunoassay. Punch biopsies of 6 mm were taken from lesional skin of 10 psoriatic patients, lesion-free skin of 6 psoriatic patients and control skin of nine subjects, immediately frozen and stored at -80° C. Samples were extracted in 0.1 M boiling acetic acid for 10 min. and centrifuged at 11000 rpm for 20 min. Extracts were assayed by means of a specific antiserum, named AH78, recognizing the carboxy terminus VIP 22 - 28. VIP-IR levels were markedly higher in lesional skin of psoriatic patients (mean=1.10 pmol/g tissue) when compared to either lesion-free psoriatic skin (mean=0.35 pmol/g tissue) or control skin (mean=0.26 pmol/g tissue). These results suggest that an increased availability of VIP could be responsible, at least in part, for a vasodilation and thus play an important role in the inflammatory processes associated with psoriatic lesions.

EXTRACELLULAR ATP INCREASES INTRACELLULAR CALCIUM (Cai) AND INFLUENCES GROWTH AND DIFFERENTIATION OF CULTURED HUMAN KERATINOCYTES. S. Pillai and D.D. Bikle, Department of Medicine, University of California and V.A. Medical Center, San Francisco, CA.

Changes in cytosolic free calcium concentration (Cai) control differentiation of keratinocytes in culture (KC). In a variety of cell systems, extracellular ATP modulates cell functions by modulating Cai levels, mediated through a purinergic receptor-operated calcium channel. In the present study, we evaluated the effect of ATP on Cai, growth and differentiation of KC. ATP increased the Cai of KC in a rapid and transient fashion, an effect that was observed both in the presence of calcium (1.2 mM) and in its absence (1 mM EGTA). Half maximal effect was observed at 30 uM ATP and saturation was achieved at 100 uM ATP. Pretreatment of cells with ionomycin abolished the ATP effect, suggesting that ATP releases Cai from intracellular sources. Single cell image analysis indicated a heterogeneity among keratinocyte populations in their response to ATP. Since 500 uM ATP did not affect cell permeability, as measured by propidium iodide fluorescence, this suggests that the effect of ATP is both specific and nontoxic. Exposure of KC to 100 uM ATP for 4 days stimulated DNA synthesis and inhibited cornified envelope formation. 100 uM ATP acutely (2 hrs) stimulated ^3H thymidine incorporation, but exerted no effect on cornified envelope formation. These studies suggest that KC possess a specific purinergic, receptor-mediated, calcium flux mechanism, and that ATP may be a regulator of epidermal growth and differentiation.

THE EFFECTS OF DERMAL PASTES ON FULL-THICKNESS WOUND HEALING IN SWINE. Louis A. Pirone, Karyn A. Monte, Ronald J. Shannon, and Laura Bolton. ConvaTec Wound Healing Institute, Princeton, NJ.

The efficacy of dermal wound pastes has not been extensively reproted in the literature. The objective of this animal study was a controlled, randomized wound healing evaluation of several wound healing pastes. The study was performed on full-thickness wounds in swine, and the method of quantification was the rate of wound contraction measured with an Optomax image analysis system. Eight, circular full-thickness wounds, 25 mm in diameter were made with a scalpel blade excising down to fascia. Each paste was applied either under an occlusive dressing, or under a non-occlusive dressing according to the manufacturer's specifications. Our results indicate that the percent contraction rate on post-operative day seven was higher for DuoDERM^R paste under DuoDERM^R CGF and Comfeel^R paste under Comfeel^R ulcer dressing, than for gauze alone, Envisan^R, Intrasite Gel, Panafil^R, Chloresium, Carrington^R Gel under gauze, and Carrington Gel^R under DuoDERM^R CGF. By post-operative day fourteen, all treatments had higher percent contraction rates compared to day seven, and Debrisan^R, gauze alone, Chloresium, and Panafil^R were significantly less contracted than the other treatments. The data indicate that wound healing contraction is more enhanced when dermal pastes are used under occlusive dressings than when the same pastes are used under non-occlusive dressings, and that some dermal pastes have an adverse effect on full-thickness wound healing in swine.

QUANTITATION OF INTRACELLULAR FREE CALCIUM (Cai) BY SPECTROFLUOROMETRY AND CORRELATIVE ULTRASTRUCTURAL LOCALIZATION BY ION CAPTURE CYTOMETRY IN HUMAN KERATINOCYTES. S. Pillai, and G.K. Menon. Department of Dermatology, University of California and V.A.M.C., San Francisco, CA.

Changes in Cai levels modulate growth and differentiation of keratinocytes in culture (KC). More differentiated KC in epidermis, as well as in culture, demonstrate increased Cai levels. In KC, we quantitated Cai with the fluorescent probe Indo-1, and localized Cai in different intracellular compartments by oxalate-pyroantimonate precipitation and electron microscopy. The resting Cai level of confluent KC grown in 1.2 mM calcium was 180-220 nM. Ionomycin increased Cai levels to maximum (uM range) which were sustained in the presence of extracellular calcium (Cao). In the absence of Cao (1mM EGTA), ionomycin evoked a transient increase in Cai (600-800 nM) within seconds, by release from intracellular sources. A new equilibrium of Cai (30-40 nM) was achieved within 5 min. In the cytochemical technique, cells were heterogeneous in their calcium content, and the calcium precipitate appeared as electron dense granules. Mitochondria, endoplasmic reticulum (ER) as well as phagocytic vesicles contained precipitates. Unlike intact epidermis, KC did not show appreciable quantities of intercellular calcium. The quantities of calcium precipitate increased significantly by ionomycin treatment in the presence of Cao, in agreement with the fluorescence data. In the presence of ionomycin, EGTA caused a depletion of Cai from the ER, but the Cai of the vesicular compartment was retained. These studies indicate a correlation between the two methods and validate the usefulness of the cytochemical technique for calcium localization in cultured cells.

PHORBOL ESTER REGULATION OF HUMAN KERATINOCYTE PROLIFERATION AND DIFFERENTIATION. M.R. Pittelkow, R.J. Coffey, Jr., Department of Dermatology, Mayo Clinic, Rochester, MN and Department of Medicine, Vanderbilt University, Nashville, TN.

Active phorbol esters exhibit pronounced effects on both epidermal proliferation and differentiation when applied to skin. Differentiation is accelerated and the germinative cell population of epidermis is markedly expanded. Mechanisms mediating the differential regulation of these opposing epidermal responses by phorbol esters are unknown. We investigated the early and delayed effects of phorbol-12-myristic-13- acetate (PMA) on cell growth kinetics, expression of transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) receptor, and differentiation of normal human keratinocytes (NHK) grown in serum-free medium MCDB 153. Early responses (2-12 hr) to PMA (10-100 ng/ml) treatment include enhanced expression of TGF-α but marked decrease in transcription of EGF receptor and c-myc and inhibition of ligand binding to EGF receptor. G_1 and G_2 growth arrest occurs in a large population of NHK and a select cell subpopulation is induced to terminally differentiate. Prolonged exposure to PMA (1-6 days) causes transient down-regulation of TGF-α transcription followed by recovery of TGF-α and EGF receptor expression, resumption of DNA synthesis and replication in the majority of NHK. The differential responses are dependent on specific subpopulations of NHK that arrest growth and differentiate or resume proliferation and express TGF-α, an autocrine growth factor and EGF receptor, the cognate receptor for TGF-α. These findings reconcile effects of phorbol ester on both epidermal proliferation and differentiation and may delineate mechanisms involved in clonal expansion of initiated cells and tumor promotion *in vivo*.

DIOXYGEN DIFFUSION IN THE STRATUM CORNEUM. William Z. Plachy, Mary E. Hatcher, Department of Chemistry and Biochemistry, San Francisco, CA.

Most of the dioxygen required by the viable epidermis must be derived from "blood oxygen" diffusing from the dermis rather than by "air oxygen" diffusing across the stratum corneum (SC). The SC thus appears to be an effective diffusion barrier to air oxygen uptake by the skin. In these experiments we measure this barrier. We have taken advantage of the paramagnetism of dioxygen, which causes dissolved dioxygen to increase the electron spin resonance (ESR) line width of our spin probes in a manner which depends on the product of the dioxygen diffusion coefficient times the dioxygen solubility. We monitor these line width effects as a function of times by observing the line intensity of the spin probe signal while in the second derivative mode of the ESR spectrometer. Dioxygen diffuses at a rapid rate in a SC lipid mixture. The apparent dioxygen diffusion coefficients for whole SC sheet are smaller than the SC lipid values by a factor of 50-to-100. The SC dioxygen diffusion coefficient is 3×10^{-8} cm^2/s. We propose that dioxygen diffuses in the SC via a lipid pathway. If the effective length of the lipid pathway is about eight times the true thickness of the SC, the tortuosity of the lipid pathway would be sufficient to account for the dioxygen diffusion rate we observe in the SC.

SUPER GLUE SAMPLING OF NORMAL STRATUM CORNEUM FOR ELECTRON SPIN RESONANCE AND ULTRASTRUCTURAL STUDIES. William Z. Plachy, Selwyn J. Rehfeld, and Peter M. Elias, Department of Chemistry and Biochemistry, San Francisco State University, and Dermatology Service, Veterans Administration Medical Center, San Francisco, CA.

Selected spin labels have been shown to be useful probes of the sample polarity, fluidity, hydration, and response to dioxygen of lipid environments in the stratum corneum (SC). Well-resolved electron spin resonance (ESR) signals can be obtained from as little as one μg of perdeuterated spin probe (pDTBN) in a few hundred micrograms of tissue. A major difficulty in studying diseased or experimentally perturbed SC has been the lack of appropriate normal SC for comparison. We obtained intact sheets of SC from normal volunteers after bonding the skin surface to a flat quartz plate (4 mm x 20 mm x 1.5 mm) with a small amount of "Super Glue" (Duro, Loctite Corporation, Cleveland, OH). The mounted SC samples then were labeled with the spin probe by vapor-phase transfer (no immersion in solvents). Electron micrographs demonstrated that the bonded SC sample appear identical to processed biopsies of normal SC, with superior preservation of intercellular dimensions. When the quartz-bonded SC samples are placed in the variable-temperature ESR dewar, signals are not observed from either the cured resin, or from the spin probe dissolved in the resin, when administered after the resin has cured. Thus, the observed ESR signals arise entirely from the spin probe in the bonded SC sample. Super glue sampling provides a superior method to obtain normal SC for studies of UV irradiation, drug penetration, desquamation, hydration effects, microscopy, or other spectroscopic investigations.

HUMAN PROTOPORPHYRIA: LONG-TERM VARIANCE IN PROTOPORPHYRIN BALANCE IN RBC AND FECAL DISTRIBUTION COMPARTMENTS IN 24 PATIENTS. Maureen B. Poh-Fitzpatrick, Department of Dermatology, New York Medical College, Valhalla, NY.

Patients with protoporphyria maintain dynamic metabolic balances for protoporphyrin (PP) levels in multiple distribution compartments that are stable for 5-7 days (Gastroenterol 88:1239, 1985), but are perturbed by liver dysfunction (Am J Med 80:943, 1986). To examine effects on this metabolic balance of the natural course of the disease in the absence of overt liver dysfunction, serial determinations of 1) PP excreted daily in feces and 2) PP burden of the estimated total rbc mass were made in 24 protoporphyria patients with normal liver function profiles over 7.1 $\pm$ 1.4 years, with 9 $\pm$ 2.5 determinations per patient. Ratios for 24h fecal PP/rbc mass PP were calculated. If hepatic efficiency for PP clearance falls over years of time, diminishing fecal PP excretion and rising rbc PP burden would be expected to yield progressively smaller ratio values. First-order regression of serial ratio values for each patient on time (months) yielded lines with positive slopes in 15 cases ($\bar{x} \pm$ SD: .0023 $\pm$ .0020, range: .0001 to .0075) and negative slopes in 9 cases ($\bar{x} \pm$ SD: -.0010 $\pm$ .0010, range: -.0001 to -.0030). Of the 9 negative slopes, 6 deviated only marginally from flat lines.

These data support the concept that little or no adverse change in liver function over several years' time, as detected by this index of hepatic PP clearance efficiency, is the natural course of human protoporphyria in the majority of patients.

CLONING OF HUMAN EPIDERMAL TYPE I TRANSGLUTAMINASE (TGASE). R.R. Polakowska, E.R. Herting, L.A. Goldsmith. Department of Dermatology, Univ. of Rochester School of Medicine and Dentistry, Rochester, NY.

Using PCR, over 65% Human Type I TGase cDNA was cloned and analyzed. A set of cDNA fragments enriched in TGase sequences was made by selective DNA amplification of a lambda gt11 human keratinocyte (HK) cDNA library. PCR amplification was driven by two primers; a pool of degenerate primers complementary to the TGase active site and another primer annealing to the 5' end of lambda gt11 vector flanking the insertion site. Amplified cDNA fragments were digested with EcoRI endonuclease to create vector-free probes for screening the library in a second step. Partial sequence analysis of one of the positive clones revealed an 18 nucleotide active site sequence identical to that of guinea pig liver TGase (Type II) and rabbit tracheal epithelium (RTE) TGase I. Two mismatches in the third nucleotide position of the glutamine and valine codon were found when compared to human factor XIIIa TGase. The overall homology of 959 nucleotides of the clone was 65% to the guinea pig liver TGase, 62.2% to human factor XIIIa and 90.6% to the RTE type I enzyme. Northern blot analysis showed that TGase mRNA was 3.7kb in SCC-9 cells known to express type I TGase activity, in normal HK cultured in high Ca^{+2} media and absent in HK in low Ca^{+2} media. Retinoic acid suppressed the mRNA in SCC-9 and in HK. The TGase transcript was not detectable in SCC-4 cells expressing only type II tissue TGase, in human fibroblast or in liver cells. Based on high homology to the RTE TGase I, the distribution in SCC lines, the response to retinoids and calcium, the differences compared with factor XIIIa and tissue TGase, and the mRNA size we consider this clone to be human type I TGase. The 90% sequence homology of human epidermal enzyme to the rabbit enzymes suggests that the gene is highly conserved during evolution and the specialized functions of TGase I tolerates few structural changes.

AGE-RELATED CHANGES IN CONTACT HYPERSENSITIVITY. J. Potozkin, N.A. Soter, and D.V. Belsito. Department of Dermatology, New York University Medical Center, New York, NY.

This study further evaluates the deficient contact hypersensitivity (CH) response of aged mice. Aged (16-18 mos.) and young (3-5 mos.) BALB/c mice were treated with: (1) IL-2, 250 U, IP, BID from days 0-2; (2) 2.5×10^7 T cells from naive young mice, IV x 1 on day 6; or, (3) cimetidine, 100mg/kg, IP, BID from days 0-2. All animals except irritant controls were sensitized with 0.1% 2,4,6-trinitro-1-chlorobenzene (TNCB) on day 0. On day 7, all mice were challenged with 1% TNCB to one ear. Ear swelling responses, given in units x 10^{-2}mm, were evaluated in each group 24 h after challenge. IL-2 [which not only enhances T cell function but also upregulates the density of Ia^+ Langerhans cells (LC) in aged (from 426$\pm$46 to 748$\pm$56 Ia^+ LC/mm^2) and young mice (from 698$\pm$27 to 900$\pm$19)] improved CH in the aged (IL-2: 22.2$\pm$0.8; saline: 5.2$\pm$0.8, $p<0.001$), although it did not normalize the response when compared to IL-2-treated young (29.7$\pm$1.5, $p<0.005$). In contrast, naive young T cells administered 24 h prior to challenge did normalize CH in the aged (T cells: 18.6$\pm$1.1; saline: 9.6$\pm$1.4, $p<0.001$) when compared to similarly treated young mice (22.8$\pm$2.0, $p=0.1$). As opposed to its enhancement of CH in young mice via inhibition of T_S induction during sensitization, cimetidine had no effect on CH in aged mice (cimetidine: 1.1$\pm$1.6; saline: 1.25$\pm$1.4, $p>0.9$). We conclude that neither the reduced density of Ia^+ LC nor the induction of T_S play a major role in the diminished CH of aged mice. The primary deficiency in CH in the aged seems related to T cell function, most likely the production of IL-2.

THE BIOPHYSICS OF WATER TRANSPORT THROUGH STRATUM CORNEUM.

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In spite of profound compositional and morphological differences between the lipids of the SC and cell membranes, the biophysics of water transport through each is remarkably similar. In contrast, the water permeability (P) through SC is about 1000-fold less than through cellular membranes. Several investigators have suggested that the unusual lipid composition of the SC is responsible for this very low P. This is unlikely however, in light of the mechanistic similarity between the permeability properties of SC and other lipid biomembranes. In contrast, the unique morphology of the SC may contribute to the exceptionally low P in this tissue. Evidence will be presented showing that water transport through the SC is characterized by a diffusion pathlength which is more than 100-fold greater than the sample thickness. These results suggest a highly tortuous permeation path and are consistent with transport primarily through the extracellular lipid domains. Thus, the "brick and mortar" structure of the SC may contribute significantly to barrier properties by requiring water to traverse a highly tortuous path.

SELECTIVE INHIBITORY EFFECTS OF BUTHIONINE SULFOXIMINE AGAINST HUMAN MELANOMA. Joseph Prezioso, George B. FitzGerald, and Michael M. Wick, Dana-Farber Cancer Institute and Dept. of Dermatology, Harvard Medical School, Boston, MA.

We have shown that buthionine sulfoximine, BSO, a selective inhibitor of *de novo* glutathione synthesis, is cytotoxic to melanoma cells (Pig. Cell Res. 2:41, 1989). Our initial studies suggested that BSO cytotoxicity was related to the levels of tyrosinase, since cell lines that had low tyrosinase levels were not affected by BSO and conversely cells with high tyrosinase were sensitive. However, one melanoma cell line, RPMI-7951, containing low tyrosinase levels was sensitive to BSO. Furthermore, normal melanocytes which have high levels of tyrosinase activity were not as sensitive to BSO as less pigmented melanoma cells.

Cell Line	Tyrosinase Activity (cpm/5x10^{5}cells)	ID_{50} μM
SK-MEL-30	8775	18
SK-MEL-28	241	>1000
RPMI-7951	178	85
M191 (normal)	4903	531

When cells were treated with PTU, an inhibitor of tyrosinase, they remained sensitive to the effects of BSO, suggesting that tyrosinase was not contributing to BSO cytotoxicity. BSO does not act as a direct inhibitor, since inhibition of DNA synthesis by BSO does not occur immediately, but correlates with the decrease in intracellular levels of glutathione. Although BSO appears to be a melanoma specific agent, this specificity is not directly related to the pigment phenotype. *In vivo* anti-tumor studies with the B-16 melanoma are in progress.

BARRIER FUNCTION REGULATES EPIDERMAL DNA SYNTHESIS. Ehrhardt Proksch, Kenneth R. Feingold, Peter M. Elias, Dermatology and Medicine Services, Veterans Administration Medical Center and University of California School of Medicine, San Francisco, CA.

Prior studies have demonstrated that alterations in barrier function regulate epidermal lipid synthesis. In this study, we have examined the possibility that barrier function is linked to epidermal hyperplasia by several acute and chronic murine models of barrier disruption. Epidermal injury by tape stripping (large increase in TEWL) resulted in an increase in DNA synthesis, which could be prevented by occlusion. Solvent removal of lipids by acetone (no histological evidence of epidermal injury) also produced an increase in DNA synthesis, and the degree of barrier disruption correlated linearly with the extent of the increase in DNA synthesis. Moreover, occlusion with a water vapor-impermeable Latex foil prevented further increases in DNA synthesis more effectively than did partially impermeable wraps (Duoderm and Opsite) which, in turn, were more effective than the vapor-permeable, Goretex foil. Furthermore, chronic barrier disruption (essential fatty acid deficiency, EFAD) also provoked an increase in DNA synthesis, which again could be prevented by Latex occlusion. Finally, topical applications of fatty acids which repair the barrier in EFAD animals (linoleic acid and columbinic acid) decreased DNA synthesis, while topical prostaglandin E_2 (no effect on barrier) did not significantly reduce DNA synthetic rates. These studies link epidermal barrier function directly to DNA synthesis, and show that in addition to increased lipid synthesis, DNA synthesis represents a second mechanism by which the epidermis maintains barrier function.

ACUTE VS. CHRONIC CHANGES IN BARRIER FUNCTION MODULATE EPIDERMAL HMG CoA REDUCTASE IN SPECIFIC EPIDERMAL CELL LAYERS. Ehrhardt Proksch, Kenneth R. Feingold, Peter M. Elias, Dermatology & Medicine Services, VAMC, and Depts. of Dermatology & Medicine, Univ. of California School of Medicine, San Francisco, CA.

The epidermis is a highly active site of lipogenesis, with synthesis occurring in all nucleated layers. We have shown recently that lipid synthesis and the activity of the regulatory enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA), are regulated by barrier requirements. To determine which epidermal layer(s) respond to alterations in barrier function, we quantitated HMG CoA reductase activity in the upper and lower layers of murine epidermis (prepared with either S. aureus epidermolytic toxin or 10 mM dithiothreitol) after various types of barrier disruption. Under basal conditions, 13 and 87% of HMG CoA reductase activity localized to the upper vs. lower epidermis, resp. Acetone and SDS treatment (acute disruption) increased enzyme activity by 54% and 30%, resp., in the lower layers only, a change prevented by restoration of barrier function by occlusion. In contrast, in essential fatty acid deficiency (chronic disruption) HMG CoA reductase activity was increased solely in the upper epidermis (161%), a change reversed by occlusion. Finally, prolonged occlusion of normal epidermis produced a decrease in enzyme activity, localized to the upper epidermis. These results show that: 1) HMG CoA reductase is present in all nucleated cell layers of the epidermis; 2) acute changes in barrier function stimulate enzyme activity in the lower epidermis; while 3) chronic maintenance of the barrier is mediated by changes in enzyme activities in the upper layers of the epidermis.

MD-PHDs IN DERMATOLOGY. Janet H. Prystowsky, Department of Dermatology, Columbia University, New York, New York.

A national survey was conducted to explore how MD-PhDs balance their research and clinical interests and what factors influence their career pathway decisions. A questionnaire was mailed to all MD-PhDs in dermatology as determined by a preliminary survey of dermatology training programs recognized by the American Academy of Dermatology. The survey included two questionnaires; one was directed to residents in training and the other was directed to fellows and attendings. The survey included questions about research background, career pathway, attitudes about personal and professional issues, and influence of residency training program on career decision making. From 60 fellows and attendings, 42 (70%) questionnaires were completed; of 29 residents surveyed, 26 (84%) completed questionnaires. Eighty-one percent of the fellows/attendings held positions in academic medicine; 76% entered dermatology with the intent to pursue academic medicine. Almost all (93%) held academic positions immediately after residency; 50% held positions with the title of assistant professor or higher as their first post-residency employment. Almost all residents responded that they entered dermatology with the intent to pursue academic medicine. However, 50% stated that their goals regarding post-residency plans had changed during their residency training. Seventy-seven percent of the residents compared with 50% of the fellows/attendings were completing their training with loans (mean $35,000 for current residents). Six cited favorable and six cited unfavorable faculty influence for pursuing academic medicine. Most MD-PhDs pursue academic dermatology; faculty are influential in this decision making process.

EVALUATION OF PORCINE OMENTAL EXTRACT IN WOUND HEALING USING ULTRASOUND IN NORMAL VOLUNTEERS. Peter T. Pugliese, and Federico Moncloa, Pugliese Assoc. Bernville, PA and Clinical Research Department, Angio-Medical Corp., New York, NY.

An angiogenic lipid fraction from porcine omentum (POE) was evaluated for wound healing ability. Four punch biopsies 4x4 mm were made in the lumbo-sacral region of each of 15 subjects. In a double-blind random manner, two wounds were treated daily for 28 days, and two served as untreated controls. Healing was evaluated by 1) external diameter; 2) external area on magnified acetate tracing; 3) ultrasound imaging with B-scans. The volume of the wound was evaluated from measurements of the ext. and int. diameters and the depth of the wounds on Days 1,2,3,7,10,14,17,21 and 28. Criteria of efficacy were 1) time to heal; 2) rate of closure of ext. diameter, area and volume; 3) scar evaluation. Age of subjects was 51-66 yr.

Results: Healing 20.8±0.5 for POE treated wounds vs 25.1±1.1 days for controls ($p=.002$). At day 17, the external diameter, the area and the volume were 2.5mm, 2.6mm^2 and 1.1mm^3 in the POE wounds versus 3.0mm, 3.8mm^2 and 1.5mm^3 in the controls ($p<.05$ in all cases). At Days 21, 66.7% POE treated wounds healed vs 26.7% of controls. Healing rate followed a polynomial curve different for treated and untreated wounds.

Conclusions: Older subjects are a reliable model for wound healing study. Wound volume reduction is good index of wound healing. In this model POE is an effective wound healing agent.

VITAMIN A DEFICIENCY AND DIOXIN-INDUCED CUTANEOUS TOXICITY IN MURINE SKIN. S. Madli Puhvel, Michael J. Connor and Midori Sakamoto, Division of Dermatology, UCLA School of Medicine, Los Angeles, CA.

The mechanisms involved in the induction of toxicity by 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), a prototype for a group of toxic polyhalogenated aromatic hydrocarbons, are largely unknown. Because dioxin exposure is known to significantly reduce liver vitamin A levels in experimental animals, it has been proposed that induction of vitamin A deficiency is involved in expression of dioxin-induced toxicity. We investigated the role of vitamin A deficiency in modulating the cutaneous response of congenic HRS/J haired and hairless mice to dioxin. Hairless mice express dioxin-induced cutaneous toxicity. Haired mice normally do not. Mice raised on a vitamin A deficient diet, and controls raised on standard chow, were treated topically with dioxin and their cutaneous response monitored histologically. Additional parameters of toxicity monitored were body and thymus gland weights. Liver vitamin A levels were determined by HPLC. Vitamin A depletion by itself had little effect on the normal cutaneous histology of either phenotype. In haired dioxin-treated animals cutaneous morphology was not affected even when these were severely vitamin A depleted. In hairless mice, vitamin A deficiency increased the sensitivity of the skin to dioxin-induced toxicity. Dioxin-induced body weight loss, and atrophy of the thymus gland, were not affected by vitamin A status of either phenotype. These experiments demonstrate that although vitamin A deficiency potentiates the expression of cutaneous toxicity in hairless mice, it had no impact on the cutaneous response of congenic haired mice and therefore expression of the toxic effects of dioxin clearly involves more complex mechanisms than a reduction in vitamin A levels.

NOVEL MONOCYTE ATTRACTANTS IN STRATUM CORNEUM FROM PSORIATIC LESIONS. D.G. Quinn and R.D.R. Camp, Institute of Dermatology, St. Thomas's Hospital, London, England.

Infiltrates of monocyte/macrophages are a characteristic feature of early and established psoriatic lesions. Aqueous extracts of stratum corneum from untreated lesions (n=9) and from normal heel as control (n=5) have therefore been tested for human peripheral blood monocyte attractant activity in a 48-well microassay incorporating 5μm pore-size polycarbonate filters. Dilution-related activity was detected in supernatants from both sources, maximal migration indices (MMI) being significantly greater in the psoriatic than in control samples (4.89 ± 0.93 versus 2.5 ± 0.37, mean ± s.e.m.; $p<0.05$, t test). Ultrafiltration of aqueous psoriatic stratum corneum supernatants through YM10 membranes and bioassay of filtrates and retentates revealed greater activity in the >10kD fractions than in the <10kD fractions (MMI 3.55 ± 0.52 versus 1.69 ± 0.39; data from 9 patients; $p<0.01$, t test). Reversed phase HPLC of >10kD samples and assay of 1 min fractions consistantly revealed two peaks of monocyte attractant activity, eluting at 60% and 100% acetonitrile (n=4). Repurification of the earlier eluting reversed phase HPLC peak by TSK gel filtration HPLC, and assay of fractions, showed a peak of activity eluting with a relative molecular mass of less than 13.7kD.

To our knowledge, this is the first report of the detection of such monocyte attractant activity in psoriatic lesional samples. The identity and source of the two partly purified biologically active materials remain to be determined, but they could play a central role in the induction of lesional monocyte infiltration in psoriasis.

MOLECULAR CLONING OF THE 52KD Ro ANTIGEN. Harry Ratrie III, and Thomas T. Provost, Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

We have used high resolution immunoblot procedures to screen autoimmune sera from patients with Sjögren's syndrome (SS), neonatal lupus erythematosus (NLE), and systemic lupus erythematosus (SLE). We have identified sera which are specific for the multiple Ro antigens, and we have used these sera in selecting clones for these antigens from lambda gt11 cDNA expression libraries. We have confirmed the identity of clones for both the 52 and 60kD Ro antigens by isolating affinity purified antibodies that bound to the cloned fusion proteins and showed that in immunoblot experiments these antibodies detect only the specific Ro antigens. In addition, we have tested the binding of autoantibodies from SS, NLE, and SLE patients to cloned fusion protein. Although the cloned inserts are not full length cDNAs, we find that 75% of sera which are Ro antibody positive by immunodiffusion and sandwich ELISA bind the Ro antigen clones while sera from normal controls or other autoimmune patients do not. Preliminary analysis of fusion protein binding does not show any relationship between the disease subgroups and the presence of autoantibodies to particular forms of the Ro antigen.

We have continued more detailed analysis of the 52kD Ro antigen clone and present here partial DNA sequence of the cDNA clone. This sequence has been searched for similarity to the 60kD Ro antigen, possible RNP binding consensus, and other reported functional motifs.

THE EXPRESSION OF KP-1, A MARKER FOR MONONUCLEAR PHAGOCYTES (MP), IN NORMAL AND INFLAMED SKIN. V. Rae, F. Jimenez-Acosta, N. Penneys, Department of Dermatology, Univ. of Miami, Miami, Florida.

KP-1 is a monoclonal antibody that recognizes a 110 Kd. protein in cells of MP lineage in formalin-fixed paraffin-embedded sections but does not recognize accessory cells such as Langerhans cells. We have examined the distribution of KP-1+ MP in normal and inflamed skin.

The avidin-biotin-peroxidase method was used to examine the expression of KP-1 in 6 micron sections. Appropriate controls were included in each experiment. A variety of inflammatory conditions were studied.

In normal skin, KP-1+ MP were scattered throughout the dermis and subcutaneous fat but never within the epidermis or adnexal structures. KP-1+ MPs were increased around blood vessels and in the vicinity of eccrine gland coils and ducts, and surrounding pilar units. KP-1+ MPs were scattered within inflammatory infiltrates, particularly granulomas and increased around hair follicles in alopecia areata.

We conclude that: 1) dermal dendritic cells which appear monomorphous with hematoxylin and eosin staining represent in fact a heterogeneous population, 2) KP-1+ MP have a unique distribution, different from that of other dendritic cells found within the dermis, with accentuation around adnexal structures, and 3) KP-1+ MPs may have a role to play in the pathogenesis of alopecia areata.

THE EFFECTS OF 1,25-DIHYDROXYVITAMIN D_3 AND RETINOIC ACID ON SOLUBLE AND PARTICULATE TRANSGLUTAMINASES IN CULTURED HUMAN KERATINOCYTES: RETINOIC ACID BLOCKS 1,25-DIHYDROXYVITAMIN D_3 ACTIVITY. Swapna Ray and Michael F. Holick. Vitamin D Laboratory, Boston University School of Medicine, Boston, MA 02118.

Transglutaminases (TGases) are a class of Ca^{2+}-dependant enzymes which are known to exist in two forms. The detergent-soluble particulate variety is responsible for cornified envelope formation during the terminal differentiation of epidermal keratinocytes, while the function of the soluble "tissue" TGase is unknown. We have separated the two forms of TGases from cultured human keratinocytes, and studied their modulation by $1,25(OH)_2D_3$ and retinoic acid (RA). TGase activities were measured by the cross-linking of ^{3}H-putrescine with casein. 24 Hours after its addition to cultured keratinocytes, $1,25(OH)_2D_3$ (10^{-8}M) increased the soluble and particulate TGase-activities by 120±3% and 138± 8% respectively over the control. On the other hand, when cultured keratinocytes were exposed to RA (10^{-6}M) for 24h, there was marked inhibition in both soluble (60±0.1%) and particulate (62±2.5%) TGase activities. To determine what effect RA would have on the TGase-stimulatory activity of $1,25(OH)_2D_3$, cultured keratinocytes were exposed to 10^{-8}M of $1,25(OH)_2D_3$ and 10^{-6}M of RA. We observed that RA completely blocked the stimulatory effect of $1,25(OH)_2D_3$ on both forms of TGase activities. Hence, these data provide a new insight into the physiologic action of RA and $1,25(OH)_2D_3$ on TGase-activity, and explain why RA inhibits, while $1,25(OH)_2D_3$ enhances the cornified envelope formation. The mechanism by which RA inhibits the $1,25(OH)_2D_3$-induced TGase-activity will be discussed.

MONOCLONAL ANTIBODIES DERIVED FROM BASAL CELL CARCINOMA (BCC) REACT WITH BCC STROMA, PAPILLARY DERMIS AND THE FIBROUS SHEATH OF THE FOLLICLE. S Ramon y Cajal, R Paus, K Stenn, D Leffell. Department of Dermatology, Yale University School of Medicine, New Haven, CT, USA.

To study epithelial-mesenchymal interactions, we are searching for markers of early hair follicle growth. Since earlier work has shown that BCC has some features of the primitive hair follicle, we attempted in these experiments to produce monoclonal antibodies to BCC, which might cross react with the follicular tissue. Basonodular BCC were collected at surgery and injected s.c. with adjuvant in Balb/c mice following standard immunization protocol. Spleen cells of the mouse with the highest blood titre to BCC were fused with cells from a mouse myeloma cell line (P3X63Ag8) and cloned twice. Two monoclonal antibodies were collected: BCC-3 and BCC-4. Both antibodies reacted with the BCC stroma exclusively: BCC-3 stained specific cells in the stroma strongly and the tumor itself lightly. BCC-4 stained the BCC stroma throughout strongly. In normal skin, BCC-3 stained dendritic cells about the follicles and in the dermis at all levels. BCC-4 stained the stroma of the fibrous sheath of the follicles as well as the upper papillary dermis most strongly. Both antibodies stained the basal membrane of eccrine ducts. The BCC-3 reacted as well with the apical membrane of eccrine ducts. These antibodies may enhance our understanding of the role of the mesenchyme in BCC and the biology of hair growth. They underscore common features of BCC stroma, perifollicular connective tissue and the papillary dermis. Molecular characterization of the antigens is underway.

CYTOPLASMIC SYNTHESIS AND TRANS-CELL WALL SECRETION OF ACID PROTEINASE, A VIRULENCE FACTOR, IN SELECTED SWITCH PHENOTYPES OF *CANDIDA ALBICANS*. Thomas L. Ray and Candia D. Payne, Dept. of Dermatology, University of Iowa College of Medicine, Iowa City, IA.

Candida acid proteinase (CAP), a keratinolytic enzyme involved in corneocyte adherence and invasion, is secreted by pathogenic *Candida* spp. CAP was localized by immunogold ultrastructural study of *C. albicans* strain WO-1 cells 99% pure for phenotype **W** (CAP-) or **O** (CAP+) and mixed phenotypes of the CAP+ strain Val-1. Cells, grown in BSA-Remold media, were fixed, sectioned and coated with goat sera, then labeled with specific mouse anti-CAP sera (1:100) and stained with 1 to 10 nm gold conjugated-goat anti-mouse IgG sera. Cells were viewed by TEM after light osmium, uranyl acetate and lead citrate staining.

CAP was observed in Val-1 blastoconidia and phenotype **O** of strain WO-1. Phenotype **W** lacked CAP and no preformed enzyme was stored. CAP was seen on cytoplasmic microtubular elements leading to the plasma membrane, and in discrete clusters and columns within the cell wall suggesting trans-cell wall secretion channels. CAP was randomly distributed on fimbriae of the yeast cell surfaces, while budding/germ tube cells of Val-1 had increased intracellular and cell wall CAP over apical regions. Argon laser confocal fluorescence imaging of murine infections using FITC-conjugated antisera demonstrated CAP circumjacent to hyphal elements in the stratum corneum, supporting a role for CAP in cutaneous invasion.

After cytoplasmic synthesis, CAP is secreted through cell wall channels, decorates the cell surface and accumulates in the stratum corneum at sites of hyphal invasion. CAP antigen is a marker of phenotype **O**, but not **W**, of WO-1. **W** does not express CAP antigen. CAP is functionally linked to phenotype switching phenomena, possibly at transcription or translation, but not at secretion.

GLUTATHIONE S-TRANSFERASE(S) IN HUMAN AND RODENT SKIN: MULTIPLE FORMS AND SPECIES SPECIFIC EXPRESSION. Haider Raza, David L. Allyn, Yogesh C. Awasthi*, M. Tarif Zaim, and Hasan Mukhtar, Department of Dermatology, Case Western Reserve University and VAMC, Cleveland, OH and *University of Texas Med. Branch, Galveston, TX.

Glutathione S-transferases (GSTs) are a family of multifunctional enzymes present in tissue cytosol that catalyze the reaction between reduced glutathione and a variety of endogenous (such as the metabolism of leukotriene A_4 to leukotriene C_4) and exogenous electrophiles and are important in preventing toxic injury in a variety of tissues. Several extracutaneous tissues exhibit a distinct spectrum of isozymes that are expressed in a highly controlled fashion. Despite the fact that the skin is continuously exposed to numerous injurious agents, little is known about the expression of GST isozymes and their role in metabolism and detoxification in cutaneous tissue. In this study using specific polyclonal antibodies to the alpha, mu, and pi classes of GSTs we identified their expression in rat, mouse and human skin cytosol. In each species 1-chloro-2,4-dinitrobenzene, benzo(a)pyrene 4,5-oxide, styrene 7,8-oxide, leukotriene A_4 and ethacrynic acid were metabolized by GSTs whereas bromosulfophthalein and cumene hydroperoxide were not. Western blot analysis indicated the predominant expression of the pi isozyme in all three species. The alpha class of isozyme(s) was present only in human skin whereas the mu class of isozyme(s) was detected in rat and mouse skin but not in human skin. In situ localization using immunohistochemical techniques confirmed Western blot data. In rodent and human skin the major isozyme(s) (pi) was predominantly localized in sebaceous glands. Our data show that in mammalian skin GSTs exist in multiple forms and show species specific expression.

CULTURED KERATINOCYTE SHEETS ENHANCE EPIDERMAL REGENERATION IN A DERMAL EXPLANT MODEL. Sigrid Regauer and Carolyn Compton. Department of Pathology, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA.

Porcine dermal explants were used to test cultured keratinocyte sheets and topical mitogens for their ability to enhance epidermal wound healing. Middermal explants obtained from paravertebral areas of Yorkshire pigs with a Padgett's dermatome were kept in serum-free Dulbecco-Vogt modified Eagle medium with 20 ng/ml hydrocortisone at the air-liquid interface in multiwell Petri dishes. Cultured human keratinocyte sheets, cholera toxin, and human recombinant TGF-α and EGF were applied topically to the explant surface at day 0. Medium was changed every 3 days. Explants were harvested at days 4-6. Morphometric analysis of perifollicular epithelial outgrowth was performed from photomicrographs of explant surfaces to quantify stimulation of re-epithelialization. Cultured keratinocyte sheets promoted regeneration of a confluent epidermis within 6 days, whereas topical mitogens or serum-supplemented medium failed to do so. Among the growth factors tested, TGF-α (10 nM) was the most effective, resulting in 50% epithelial confluence at 6 days. Cholera toxin (1 nM) was less effective. EGF (2 nM) failed to promote outgrowth significantly above the serum-free baseline. The failure of human EGF to stimulate porcine explants may be due to species differences in EGF receptors. We conclude that topical application of cultured human keratinocyte sheets induce endogenous re-epithelialization more effectively than TGF-α, EGF or cholera toxin.

MOLECULAR CLONING OF DESMOGLEIN. Linda Razsi, Karen Raynor, Yigal Aharon, Motomo Manabe and Pamela Cowin. Departments of Dermatology & Cell Biology, NYU Medical Center, New York, NY.

Desmosomes are specialized domains of the plasma membrane which form sites of strong intercellular adhesion. Three major components desmoglein, desmocollin I and desmocollin II contribute towards this adhesive mechanism. One of these, desmoglein, has been implicated as the antigenic target of antibodies found in the sera of patients with the blistering disease Pemphigus Foliaceus. We have isolated eight cDNA and 2 identical genomic clones using specific oligonucleotide probes derived by PCR techniques. Together these clones encode desmoglein. Their characterization is based on identity between protein sequence derived from the cDNAs and a 40 amino acid stretch obtained by Edman degradation of a proteolytic cleavage fragment of desmoglein.

Analysis of the protein sequence reveals several highly conserved motifs found among member of the cadherin family of calcium dependent cell adhesion molecules. Northern blots show the cDNAs to hybridize with a 6.5kb mRNA in bovine snout and mRNAs of slightly different sizes in other epithelia. A single band was detected in genomic Southern blots. These data suggest that desmoglein is encoded by a single gene which is processed into multiple transcripts.

EXPRESSION OF MATURATION MARKERS IN SKIN REGENERATED FROM CULTURED EPITHELIAL AUTOGRAFTS. Sigrid Regauer and Carolyn Compton. Department of Pathology, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA.

The expression of differentiation markers in skin regenerated from cultured keratinocyte autografts of axilla-, groin-, scalp- or sole-skin origin was studied in 14 pediatric patients treated for massive full-thickness burns or giant congenital nevi. Biopsies of regenerated skin from 1 week to 3 years postgrafting were stained immunohistochemically with antibodies to involucrin, filaggrin, and the hyperproliferative keratins AE-1 and AE-3. By one week postgrafting, involucrin expression was seen in the suprabasal epidermal strata of cultured skin, and filaggrin staining was present in the stratum granulosum, as seen in uninjured skin. These patterns were maintained for the length of the observation period. Both AE-1 and AE-3 keratins were expressed in cultured skin up to 6-12 months after grafting, fading slowly thereafter. Reduction in hyperproliferative keratin expression coincided with the appearance of rete ridges in cultured skin. Autografts derived from sole skin returned to normal hyperproliferative keratin expression sooner than autografts derived from other body sites.

DISTRIBUTION OF CYTOSKELETAL PROTEINS IN EMBRYONIC AVIAN EPIDERMIS. Wende R. Reenstra, Kathy K.H. Svoboda, Department of Anatomy, Boston Univ. School of Medicine, Boston , MA.

The epidermis was separated from the underlying dermis using Dispase II. The optimal concentration and incubation period was determined for isolation with and without the basal lamina (BL). The sheets of epidermis maintain their apical-basal polarity and cell-cell junctions and are two cell layers thick. These experiments have shown that when epidermis is isolated without the BL the basal cell surface forms blebs and the actin cortical mat is disrupted. The cells respond to extracellular matrix molecules by reorganizing the basal actin cortical mat, the blebs retract, RER and Golgi are seen in abundance. Previous studies have shown that 6 day epidermal cells have an organized actin cortical mat. In the current study, using confocal image analysis (Biorad MRC 500) we investigated the distribution of cytoskeletal proteins in whole mount preparations of freshly isolated and cultured avian epidermis. The distribution of the fluorescently tagged structures were localized utilizing optical sectioning and image enhancement. Epidermal sheests were isolated from the dermis with or without the BL intact, placed on black polycarbonate filters (Poretics), then incubated with the appropriate marker. Actin was labeled with rhodamine phalloidin. Cytokeratin was visualized with a primary antibody then a fluorescently tagged secondary antibody. The actin was prominent as an organized network (actin cortical mat) in the basal compartment of the basal cells when isolated with the BL intact, and at the interface between the basal and periderm cells. Actin also defined the cell borders and microvilli of the periderm cells. Epidermis isolated without the BL did not have an organized actin cortical mat, rather the actin was localized in the blebs on the basal cell surface. Epidermis isolated without the BL intact, and cultured in the presence of extracellular matrix molecules (frozen killed lens capsules) reorganized the actin mat. Whereas, epidermis isolated without BL and cultured without BL proteins continued to have basal cell blebs. The cytokeratin filaments formed a basket-like network around the nuclei of the periderm cells, and appeared to be aligned from cell to cell. In conclusion, the use of confocal analysis has allowed us to dectect the changes in cytoskeletal protein distribution associated with the absence or presence of extracellular matrix molecules.

EPIDERMAL ORIGIN OF CUTANEOUS ANCHORING FIBRILS. Sigrid Regauer, Gregory R. Seiler, and Carolyn C. Compton. Department of Pathology, Massachusetts General Hospital and Shriners Burns Institute, Harvard Medical School, Boston, MA.

Anchoring fibrils are essential structural elements of the dermoepidermal junction and are crucial to its functional integrity. They are known to be composed largely of type VII collagen, but the cellular origin of their collagenous components has not yet been confirmed. Cultured human keratinocyte sheets were transplanted to nondermal wound beds beneath the panniculus carnosus of dorsal skin flaps in athymic mice. Anchoring fibril development was analyzed ultrastructurally during de novo dermoepidermal junction formation in vivo. Newly formed anchoring fibrils and anchoring plaques subjacent to xenografts were analyzed ultrastructurally by immunogold localization using an antibody specific for human type VII collagen. At 2 days postgrafting, the xenograft was loosely attached to the subjacent stroma, and type VII collagen was localized only within the cytoplasm of basal keratinocytes. Within 6 days, a fully differentiated epidermis had developed, and basal lamina, hemidesmosomes, and immunolabelled anchoring fibrils and plaques were present. With increasing postgrafting intervals up to 42 days, immunolabelling of type VII collagen quantitatively increased. We conclude that the type VII collagenous components of anchoring fibrils and plaques are principally products of epidermal keratinocytes and that cutaneous anchoring fibril formation can occur in the absence of dermal fibroblasts.

DISTRIBUTION OF WATER IN THE STRATUM CORNEUM: NON-PENETRATION OF INTERCELLULAR LAMELLAR LIPID BILAYERS. Selwyn J. Rehfeld, William Z. Plachy, Peter M. Elias. Department of Dermatology, University of California School of Medicine; Dermatology Service, Veterans Administration Medical Center, Departments of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA.

The degree of hydration of the intercellular lamellar lipid bilayers of the stratum corneum (SC) remains unresolved. Recently, the lipid phase transitions in dried SC, determined using the electron spin resonance (ESR)-spin probe technique (J. Invest. Dermatol. 92:505A, 1989), were compared with transitions reported for x-ray diffraction of SC in excess water (White, et al, Biochemistry 27:128-135, 1988). We found no significant difference, suggesting that water does not penetrate into the lipid bilayers. In this study, using the spin probe technique, various levels of water ranging from 10% to excess) were added to dried SC and the ESR spectra recorded over the temperature range -70° to +60°C. The probe was found to dissolve into two distinct sites, one aqueous and the other essentially non-polar. Moreover, the phase transitions of the probe in the non-polar environment were found to be essentially independent of water concentration. Furthermore, below 0°C the water of hydration associated with non-lipid sites remained highly mobile, low viscosity, until approximately -30°C, where the viscosity increased drastically. These results indicate that the intercellular lipid bilayers are impermeable to water, suggesting that water diffusion through the SC may occur via alternate pathways, such as the cornified envelope, cell interior, or other non-lamellar domains within the intercellular spaces.

SUN-INDUCED FRECKLES IN YOUNG WHITE SUBJECTS. AR Rhodes, IS Albert, RL Barnhill. Department of Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, PA, and Departments of Dermatology and Pathology, Harvard Medical School, Boston, MA.

Previous studies of sun-induced and ultraviolet B experimentally induced freckles in young white adults have revealed a reduced density of melanocytes, according to cell counts in DOPA-incubated epidermal sheets. In contrast, sun-induced freckles in older white adults have revealed melanocytic proliferations. To address these differences, we reinvestigated sun-induced freckles in young subjects using DOPA-incubated vertical tissue sections. Shave biopsies of freckles and immediately adjacent unaffected skin were obtained from the upper backs of 5 white male volunteers, ages 10-23 yr, and non-freckled skin adjacent to nevomelanocytic nevi excised from the backs of 4 additional white adults, ages 18-36 yr. Melanocyte frequency was expressed as a percentage of 800 epidermal basal unit cells. We determined that each of the freckles studied consisted of intraepidermal melanocytic hyperplasia in a lentiginous epidermal pattern, with the following melanocyte frequencies compared to adjacent unaffected skin: 27.4% vs 18.5%, 20.4% vs 15.5%, 30.9% vs 18.6%, 39.1% vs 22.3% and 31.6% vs. 17.1% (2-sided $p < 0.05$, all comparisons). In non-freckled skin adjacent to the nevus specimens, melanocyte frequencies were 16.0%, 16.5%, 12.4% and 14.8%, respectively. These data suggest that at least some sun-induced freckles in young subjects are similar to solar lentigines. "Benign" melanocytic proliferations induced by sun exposure may be relevant to melanocytic neoplasia. Additional studies of sun-induced freckles in young subjects are warranted.

THE EFFECT OF ELECTRICAL STIMULATION ON THE NUMBER OF MAST CELL PROLIFERATION. J.D. Reich, A.L. Cazzaniga, P.M. Mertz, F. Kerdel, W.H. Eaglstein, Univ. of Miami Sch. of Med. Dept. Derm. Miami, FL.

Mast cell proliferation has been reported to be associated with fibrosis, keloids, and chronic ulcers. Because it reduced post-surgical scar thickness, fibrosis, we studied the effect of pulsed electrical stimulation (PES) on the proliferation of mast cells during wound healing. Partial thickness wounds (7 x 10 x 0.3mm) were made with an electric keratome on the backs of anesthetized pigs. PES wounds were treated with a direct rectangular pulsed current of 30 milliamps peak, 140 microseconds width, and 128 pulses per second. (Current density = 0.51 mA.cm^2, Duty cycle = 1.79%). The conduction medium was saline. Control wounds were treated with sham PES. Biopsies were taken on days 0-4 and on days 7 and 14. The biopsies were fixed in Carnoy's Medium and stained with Alcian Blue and counterstained with Saffarin. The number of mast cells present were counted blindly on each day and divided by the size of the biopsy specimens to give mast cells/mm^3. Significant reduction of mast cells number during healing was seen on days 1-3 but not on days 4, 7 or 14. (Data not included).

MAST CELLS/MM^3

DAY	PES	CONTROL
1	0.25*	0.47
2	0.17*	0.55
3	0.16*	0.41

*P < .05

This study demonstrates that PES can be used to reduce the density or prevent mast cells in healing skin. The possibility exists that PES can help avoid the adverse healing conditions associated with proliferation of mast cells.

GENERATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR AN ANTI-BASEMENT MEMBRANE ZONE (BMZ) ANTIBODY IN BULLOUS PEMPHIGOID (BP) SERUM. M.J. Rico, S. Bartow, R.P. Hall, Duke Univ. Medical Center, Durham, North Carolina

To further characterize the specificity of antiBMZ antibodies from patients with BP, we generated a mouse monoclonal antibody specific for an antiBMZ IgG isolated from the serum of a patient with BP. Affinity purified antiBMZ IgG, prepared by incubating BP serum with epidermal sheets of normal skin and eluting the bound IgG with acid-glycine, was used to immunize Balb/c mice. Spleen cells from the immunized mice were fused and the hybridoma supernatants with reactivity on ELISA against antiBMZ IgG, but not against pooled human IgG, or normal human IgG, were cloned. The resultant monoclonal antibody, JR3.17, is a mouse IgG, which binds to antiBMZ IgG (net OD>2.0) but not to normal human IgG (net OD=0). Using a Sepharose 4B-JR3.17 column, IgG was isolated from BP serum (7 μg/ml IgG) but not from a normal patient or a patient with pemphigus (<0.5 μg/ml IgG). This affinity purified antiJR3.17 IgG binds by indirect immunofluorescence to the epidermal side of salt split skin. Thus, the monoclonal antibody, JR3-17, is specific for an idiotype expressed on a circulating antiBMZ antibody in a patient with BP, but not present on IgG in pooled human sera, normal human serum or pemphigus serum. This monoclonal antibody may be useful in further characterizing the idiotypic specificity of antiBMZ antibodies in patients with BP.

HLA-CLASS II TYPING BY DRB1, DQB1, DQA RFLPs: DEMONSTRATION OF GENE SHARING IN SIBLINGS WITH PEMPHIGUS VULGARIS. P. B. Reohr, A. Mangklabruks, A. M. Janiga, W. Blaszak, R. Mostofi, L. J. DeGroot, K. Soltani. University of Chicago, Chicago, Illinois.

Pemphigus vulgaris is known to be associated with the HLA serotypes DR4 and DRw6. Recently the restriction fragment length polymorphism (RFLP) method using hybridization with the HLA DQB cDNA probe identified DQw1 and DQw3 as alleles which are even more highly associated with pemphigus vulgaris than are the serologically defined DR antigens. We have used the HLA-DRB1, HLA-DQA and HLA-DQB1 genes as cDNA probes for Southern hybridization with TaqI-digested genomic DNA to study 2 siblings with pemphigus vulgaris and 3 unaffected family members and have identified the RFLP patterns associated with susceptibility to pemphigus vulgaris in this Hispanic family. Both patients had positive direct and indirect immunoflourescence for pemphigus antibody. Using both RFLP patterns from the DRB chain probe and from the DQA chain probe enabled us to determine the affected sibs' haplotype and corresponding DR antigen HLA-DR4/DR3, with mother and father, DR3a/DRw8 and DR4/DRw6b respectfully, and unaffected sib DRw6b/DRw8. RFLP patterns detected with the DQB1 probe after Southern blotting showed DNA band positions which correlated with DQw3.2 allele for the affected sibs, DQw3.2/DQw1 for the father, and DQw1 for the unaffected sib. The affected sibling sharing of the haplotype HLA-DR4 and the DQw3.2 alelle lends support to prior studies of unrelated Caucasian patients that implicated DQw1 and DQw3 polymorphisms in susceptibility to pemphigus vulgaris.

INDUCTION OF ANTIGEN-SPECIFIC SUPPRESSOR T CELLS BY A SOLUBLE SUPPRESSIVE FACTOR RELEASED BY UV-IRRADIATED KERATINOCYTES. Jorge M. Rivas and Stephen.E. Ullrich, Dept. of Immunology, M.D. Anderson Cancer Center, Houston, Texas.

A single exposure to ultraviolet radiation induces a systemic suppression of the immune response to allogeneic histocompatibility antigens. The suppression is associated with the appearance of splenic alloantigen-specific suppressor T cells. How exposing the skin to UV radiation results in the induction of splenic suppressor T cells is not entirely clear. Our data suggests the involvement of a UV-induced keratinocyte-derived suppressive factor. The keratinocyte line, Pam 212 was exposed to 200 J/m^2 of UVB radiation from a single FS-40 sunlamp and cultured overnight in serum-free medium. Injecting mice with culture supernatants from UV-irradiated keratinocytes suppressed the induction of delayed type hypersensitivity to alloantigen. Injecting supernatants from non-irradiated Pam 212 cells had no suppressive effect. Antigen-specific T suppressor cells were found in the spleens of the mice injected with the suppressive supernatants. Cycloheximide treatment of keratinocytes and trypsinization of the supernatants from the UV-irradiated keratinocytes resulted in a loss of suppressive activity suggesting the involvement of a protein. The suppressive material bound to a Concanavalin A-agarose lectin-affinity column and was eluted with α-D-mannopyranoside, indicating the suppressive material is a glycoprotein. Analysis of the suppressive material and the control supernatants by polyacrylamide gel electrophoresis demonstrated a prominent band in the suppressive fractions that was not present in the non-suppressive fractions. The approximate molecular weight of the unique band was 68 kilodaltons. Thus, these data support the hypothesis that soluble factors released from UV-irradiated keratinocytes are responsible for the induction of systemic suppression following exposure to UV radaition, by demonstrating that the injection of these factors induces antigen-specific suppressor T cells.

IMMUNOPATHOLOGICAL FINDINGS IN THE SKIN AND ORAL MUCOSA OF PATIENTS WITH ENDEMIC PEMPHIGUS FOLIACEUS. EA Rivitti, J.A. Sanches, C.R. Martins and L.A. Diaz. Depts. of Dermatology, U. of Sao Paulo, Sao Paulo, Brazil and Med. College of Wisconsin, Milwaukee, WI.

Fogo Selvagem (FS) is characterized by subcorneal acantholysis and a predominantly IgG4 autoantibody response against desmoglein I (DG-1). Further, FS autoantibodies are pathogenic to neonatal mice by passive transfer studies. Despite extensive skin involvement by vesicles and erosions, the mucosal surfaces in cases of FS remain normal. Since DG-1 is distributed in all layers of skin and mucosas, we evaluated the distribution of FS autoantibodies bound in vivo to perilesional epidermis and normal oral mucosa of patients with FS.

Serum samples and biopsies from skin and mucosa were obtained from FS patients with active skin lesions (n:13) and tested by indirect and direct immunofluoresecence (IF) respectively. The IgG and IgG subclass of FS autoantibodies was determined by IF using monoclonal antibodies against IgG1, IgG2, IgG3 and IgG4. FS IgG4 autoantibodies were detected in perilesional and mucosal intercellular spaces (ICS) of all specimens tested (13/13), wheareas FS IgG2 and FS IgG3 autoantibodies were present in only 2 patients. The intensity of IF staining of the ICS of skin and mucosa was similar in individual patients. The sera of FS patients showed the following IF titers, 1:80 (n:2), 1:160 (n:3), 1:320 (n:1), negative (n:5) and not done (n:2). This study suggests either that the specificity of FS autoantibodies is heterogenous or that the subcorneal acantholysis induced by these autoantibodies is modulated by local factors unique to the epidermis or the mucosal epithelium.

T CELL RECEPTOR (TCR) TRIGGERED INDUCTION OF INTERLEUKIN-4 PRODUCING CD4+ T LYMPHOCYTES. Martin Röcken, Kai M. Müller, Jean-Hilaire Saurat and Conrad Hauser. Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland.

The type of immune response mediated by CD4+ T cells is critically dependent on the pattern of lymphokines secreted. For example, IL-2 producing CD4+ T cell clones are capable of mediating DTH whereas IL4-producing CD4+ T cells are capable of stimulating IgE synthesis by B cells. However, the signal requirements that lead to either IL2 or IL4 production by T cells are unknown. Since freshly isolated and stimulated CD4+ T cells from nonprimed mice produce IL2 and no IL4 in vitro, we chose to study the induction of IL4 production in activated T cells. Freshly isolated (>95% CD4+) T cells from nonprimed animals were activated for 48 h by ConA and syngenic 3000 r irradiated spleen cells. The cells were then washed and expanded in fresh media and recombinant IL2. After expansion for 10-12 d the cells were restimulated and their supernatants (SN) tested for IL2 and IL4 activity using CTLL cells that respond to both lymphokines together with antibodies to IL2 and IL4. T cells which were activated and subsequently expanded in IL2 alone produced large amounts of IL2 but little or no IL4. However, when expanded with IL2 plus 20% ConA SN the T cells released large amounts of IL4 but no IL2. The effect of ConA SN was inhibited by the addition of α-methylmannoside and reproduced by addition of IL2 plus ConA or IL2 plus 2C11 (a mitogenic mAb to TCR ε chain) during the expansion culture. An effect of accessory cells on the IL2/IL4 regulation could not be demonstrated since further enrichment of CD4+ T cells by positive selection and activation with pharmacologic agents (PMA plus Con A, PMA plus ionomycine) had no effect on the T cell surface triggered induction of IL4 producing T cells. These results indicate that, besides IL2, prolonged or repetitive triggering of TcR molecules (and possibly other T cell surface molecules) is capable of inducing IL4 in freshly isolated and activated CD4+ T cells. Preliminary results from single cell cultures show that CD4+ T cells are capable of switching from IL2 to IL4 production.

CIS-UROCANIC ACID INDUCED IMMUNOSUPPRESSION IS ASSOCIATED WITH A PROSTAGLANDIN-DEPENDENT MECHANISM. Lee K. Roberts, Byound-Deuk Jun and Michael Y.L. Law. Schering-Plough, Memphis, Tennessee; Chonbuk National Univ Med Sch, Chonju, Korea; Dept of Dermatol and Pharmacol, Univ of Utah, Salt Lake City, Utah.

Trans-urocanic acid (UCA) is isomerized to cis-UCA by ultraviolet radiation (UVR). It was suggested that UVR conversion cis-UCA functions as a "photoreceptor" to induce immune suppression. We have shown that prostaglandins (PG) are involved in UVR-induced immune suppression of contact hypersensitivity (CH). The purpose of this study was to determine whether cis-UCA caused suppression of CH by a PG mechanism. Trans-UCA (4 mg/ml 10% DMSO in saline) was exposed to 5 kJ/m^2 UVR (280-320 nm), which induced 60% cis-UCA conversion. Mice were injected subcutaneously with 200 ug of isomerized cis-UCA or unexposed trans-UCA 5 hrs before sensitization with 25 ul 0.5% DNFB. After 4 days they were challenged on the right ear and ear swelling responses (Units of 10^{-4} inches) were compared between UCA treated and untreated controls, 24 hrs after challenge. Control mice had 90 U of ear swelling compared to 78 U for trans-UCA (15% suppression) and 49 U for cis-UCA (49% suppression) treated mice. To determine whether PG were involved, 1 day before UCA treatments mice received a subcutaneous implant of a indomethacin containing pellet (2.5 ug indomethacin released per day). Compared to controls it was found that indomethacin had prevented cis-UCA induced immune suppression, i.e., CH of trans-UCA treated mice was 73 U and cis-UCA mice was 74 U, a level of suppression of 19%. These results support the hypothesis that UVR-induced cis-UCA is capable of causing suppression of CH, however, it mediates its suppressive effect through a PG mechanism.

LORICRIN, A MAJOR KERATINOCYTE CELL ENVELOPE PROTEIN, IS THE SULFUR-RICH COMPONENT OF DENSE HOMOGENEOUS DEPOSITS. D.R. Roop,[1] J.A. Rothnagel,[1] M.A. Longley,[1] D. Bundman,[1] M.E. Bisher,[2] A.C. Steven,[2] and P.M. Steinert,[2] Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX[1]; NIAMS, NIH, Bethesda, MD.[2]

Considerable data suggest that cell envelopes have a high cystine content. Furthermore, dense homogeneous deposits (DHD), which have a sulfur-rich component, are thought to participate in the formation of cell envelopes. However, to date, conclusive data identifying this cystine-rich component have not been reported. We have recently isolated and characterized a cDNA clone encoding loricrin, a previously undescribed protein which is a major component of cell envelopes. The determination of the complete nucleotide sequence of this cloned cDNA has revealed that loricrin has a high cystine content (6.9%). In addition, indirect immunofluorescence studies with antibodies elicited against a specific C-terminal peptide indicate that loricrin is initially localized in granules in the stratum granulosum of mouse epidermis. These data suggest that loricrin may be the sulfur-rich component of DHD. We have now obtained electron microscopic immunocytochemical data in support of this conclusion. Affinity-purified antibodies elicited against specific peptides of loricrin and filaggrin have been used to localize filaggrin to the large cytoplasmic granules and loricrin to small round granules (DHD) in the stratum granulosum of mouse epidermis.

IMMUNOSUPPRESSIVE EFFECTS OF CLONIDINE OF THE INDUCTION OF CONTACT SENSITIZATION IN THE BALB/c MOUSE. Michael K. Robinson and Teresa J. Sozeri, The Procter & Gamble Co., Miami Valley Laboratories, Cincinnati, OH.

The clonidine transdermal therapeutic system has been associated with a significant incidence of allergic contact sensitization. This incidence was not predicted by initial skin sensitization testing in animals or humans. One possible explanation lies in recent findings in guinea pigs that clonidine exposure could inhibit the elicitation of skin reactions to unrelated strong contact sensitizers. However, these studies also showed that clonidine pretreatment did not appear to affect the induction of contact sensitization. On this basis we sought to specifically evaluate the induction phase of sensitization to clonidine as an alternative means of assessing its sensitization properties. The method selected was the assay of *in situ* lymphocyte proliferation in lymph nodes draining the sites of clonidine exposure, a method recently promoted as an alternative means to assess contact allergenic potential. Utilizing various induction application techniques, we were consistently unable to demonstrate clonidine's allergenic potential through such an assessment of lymphocyte proliferation. We were also unable to demonstrate sensitization by *in vivo* ear swelling or *in vitro* lymphocyte blastogenesis assay techniques. However, a subsequent evaluation of the effect of clonidine exposure on the induction of sensitization to unrelated strong contact allergens demonstrated a consistent 40-50% inhibition of the proliferative response to the contact allergens oxazolone and trinitrochlorobenzene. In addition, we observed a comparable inhibition of the ear swelling response to oxazolone. These data extend our knowledge of the immunomodulatory effects of clonidine and offer additional mechanistic insights into the failure of short term predictive patch test methods to detect this chemical's potential to induce allergic contact sensitization.

ULTRAVIOLET B RADIATION INDUCTION OF ORNITHINE DECARBOXYLASE GENE EXPRESSION IN THE MOUSE Cheryl F. Rosen, Dragan Gagic and Jia Qi, Department of Medicine, University of Toronto, Toronto, Canada.

Ultraviolet radiation (UVR) induces the expression of several genes, and the synthesis of their corresponding proteins. Ornithine decarboxylase (ODC) enzyme activity has been shown to increase in murine skin irradiated with ultraviolet B (UVB) in vivo and in rat keratinocytes in vitro. To study the effect of UVB on ODC gene expression, Skh hairless mice were either sham-irradiated or irradiated with increasing doses of UVB (45, 90, 180 and 270 mJ/cm^2). Northern blot analysis of epidermal RNA under basal conditions revealed the presence of two ODC mRNA transcripts. A dose-dependent increase in the levels of both ODC mRNA transcripts was noted, with the greatest increase noted with 90 mJ/cm^2. To determine the time course of the UVB-induction of ODC mRNA, total epidermal RNA was isolated at various timepoints from mice which had been exposed to 90 mJ/cm^2 UVB and Northern blot analysis performed. The induction of ODC mRNA was noted at 2h, with the maximal increase seen at 6h. No significant change in actin mRNA levels was noted under identical conditions, suggesting that the induction of ODC gene expression was relatively specific. Mice were injected with cycloheximide, a known protein synthesis inhibitor, 4 h prior to exposure to 90 mJ/cm^2 UVB. Northern blot analysis revealed that cycloheximide did not alter the UVB-induced increase in ODC mRNA. In contrast to the four-fold induction of ODC mRNA, UVB induced ODC enzyme activity 20 to 30 fold. These results raise the possibility that UVB may modulate ODC enzyme activity by both the induction of ODC gene expression and mRNA translation in murine epidermis.

A HUMAN KERATIN I GENE DISPLAYS AN ALTERED PATTERN OF REGULATION IN RESPONSE TO CALCIUM AND RETINOIC ACID IN CULTURED TRANSGENIC MOUSE KERATINOCYTES. D. Rosenthal, P. Steinert, C. Huff, S. Chung (1), S. Yuspa, and D. Roop (2), NCI, (1) USUHS, Bethesda, Maryland, and (2) Baylor College of Medicine, Houston, Texas.

Keratin K1 is one of the first markers expressed in the differentiating mammalian epidermis. In cultured mouse keratinocytes, a sharp peak for induction of K1 and other markers of terminal differentiation occurs when the concentration of Ca^{2+} in the medium is between 0.10 mM and 0.16 mM. Retinoic acid (RA) is also a strong modulator of differentiation in epidermal cells; K1 is suppressed by micromolar concentrations of RA. Primary epidermal cell cultures from newborn transgenic mice containing a 12 kb human K1 (HK1) genomic fragment were established to examine the response of the human transgene as well as the endogenous mouse K1 (MK1) to Ca^{2+} and RA. Ca^{2+} induced both HK1 and MK1, although the optimal concentration for HK1 was 0.6 mM, compared to 0.12 mM for MK1. Pretreatment of cells with different concentrations of RA down to 10^{-8}M completely inhibited MK1, while there was no detectable inhibition of HK1 at concentrations of RA up to 10^{-6}M RA. These data suggest that the 12 kb human K1 clone contains regulatory elements responsive to Ca^{2+}, while RA-responsive elements lie outside the 12 kb region.

ABNORMAL PROCESSING OF TRANSFECTED PLASMID DNA IN CELLS FROM PATIENTS WITH CHROMOSOME BREAKAGE SYNDROMES Thomas M. Rünger and Kenneth H. Kraemer Dermatology Department, University of Würzburg, West-Germany; and National Cancer Institute, Laboratory of Molecular Carcinogenesis, Bethesda MD .

Bloom´s syndrome (BS) and ataxia telangiectasia (AT) are two chromosome breakage syndromes associated with a high cancer risk. We reported a reduced and error-prone *in vivo* joining of linear plasmid DNA in BS cells (EMBO 8, 1419-25, 1989) and have now extended our studies to AT and xeroderma pigmentosum (XP) cells. We assessed *in vivo* DNA processing, spontaneous mutability, and DNA joining efficiency and accuracy of the host cells on a molecular level by passing circular or linear plasmid pZ189 through fibroblast or lymphoblast host cells and characterizing recovered plasmids afterwards. Bloom´s syndrome cells had a 3.9-5.4 fold higher spontaneous mutation frequency than normal cells and a different spectrum of mutations. In AT lymphoblasts it was 2.8 fold higher and normal in AT fibroblasts with a distinct mutational spectrum in both cell lines. XP cells could not be distinguished from normal cells. Joining of DNA ends was 2.2 -2.4 fold less efficient ($p<0.05$) in BS and AT fibroblasts, normal in XP fibroblasts. The mutation frequency of the re-joined plasmids, reflecting accuracy of intracellular DNA joining, was 52% after joining in BS cells, a value 1.4 - 1.8 fold higher than in normal cells ($p<0.001$), and 1.6 - 2.0 fold higher after passage through AT cells ($p<0.001$), normal in XP fibroblasts. Detailed analysis of 463 recovered plasmids revealed an increase of point mutations and complex mutations in BS cells, of deletions and complex mutations in AT cells, and a normal mutational spectrum in XP fibroblasts. The fact that AT cells demonstrated a different spectrum of mutations in the re-joined plasmids might indicate, that a different DNA modifying enzyme is responsible for the impaired DNA joining in AT. These abnormalities might be related to the increased chromosome breakage in cells and the high cancer risk in patients with these two genetic disorders.

DEVELOPMENT OF AN EPIDERMAL-SPECIFIC EXPRESSION VECTOR FOR TARGETING GENE EXPRESSION TO THE EPIDERMIS OF TRANSGENIC MICE. J.A. Rothnagel, D.A. Greenhalgh, M.A. Longley, D. Bundman, A.M. Dominey, and D.R. Roop, Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX 77030.

The ability to specifically target gene expression to the epidermis of transgenic mice offers the exciting possibility of creating animal models of certain skin disorders that are inherited in man. In previous studies, we have produced transgenic mice, using a 12 Kb fragment containing the human keratin 1 (HK1) gene, which express the human gene in a tissue and developmental specific manner. The expression of the HK1 gene is not identical to the endogenous mouse K1 (MK1) gene with respect to differentiation state, in that the HK1 gene is expressed in a large proportion (30-40%) of basal cells, whereas the MK1 gene is only expressed in rare basal cells (3-5%). Thus, the HK1 fragment used to produce these transgenics is lacking sequences that are required for host factors to completely suppress HK1 expression in cells with proliferative potential. We have exploited these expression characteristics to produce an epidermal-specific targeting vector by removing the keratin-coding sequences of the 12 Kb HK1 fragment. We have incorporated a poly-linker sequence in the vector to allow for the easy introduction of other sequences into the expression vector. Using ß-galactosidase as the reporter, we have successfully tested the HK1 epidermal-specific expression vector in cultured primary keratinocytes. Studies using the expression vector in transgenic mice are currently underway.

LYMPHOCYTE SUBGROUPS AND EXPRESSION OF ENDOTHELIAL CELL ADHESION MOLECULES IN THE SKIN OF PATIENTS WITH SYSTEMIC SCLEROSIS. M.H.A. Rustin, M. Kunaver*, G. Cambridge*, D.O. Haskard† and Pauline M. Dowd, Departments of Dermatology and *Rheumatology, University College and Middlesex School of Medicine, London and †Rheumatology Unit, United Medical and Dental Schools, Guy's Hospital, London.

The density of the dermal inflammatory cell infiltrate in patients with systemic sclerosis (SS) appears to correlate with the extent and progression of cutaneous sclerosis. The crucial step for leukocyte migration is interaction with endothelial cells and this involves the binding of leukocyte adhesion molecules to their ligands. In this study subpopulations of lymphocytes and expression of two such ligands on the dermal microvasculature of patients with SS were examined.

Skin biopsies were taken from involved digital and from clinically normal skin on the arms of 6 patients with SS. Similar biopsies were taken from 10 age- and sex-matched normal volunteers (V). Immunohistochemical staining was performed with the mouse anti-human mAbs UCHT-1 (CD3), Leu 3a (CD4), UCHT-4 (CD8), UCHL-1 (CD45RO), 2H4 (CD45RA), 6.5B5 (ICAM-1) and 1.2B6 (ELAM-1).

There were decreased CD3+ ($p < 0.01$) and CD8+ ($p < 0.05$) but increased CD45RO+ T lymphocytes ($p < 0.01$) in the involved skin of patients with SS compared to V using student's t-test. There was no difference in the number of dermal blood vessels expressing ICAM-1 and ELAM-1 in the involved skin but there was a decreased number and ICAM-1+, ELAM-1+ ($p < 0.05$) blood vessels in the uninvolved skin compared to V. A photodetection method to assess staining intensity showed a significantly greater expression of ELAM-1 in involved skin ($p < 0.001$).

This study has shown an increased number of 'memory', a decreased number of cytotoxic/suppressor T lymphocytes together with upregulation of ELAM-1 expression in the involved skin of patients with SS. This evidence of endothelial cell activation suggests a role of IL-1 or TNF-α in the pathogenesis of this disease.

PREDICTIVE VALUE OF PROGNOSTIC VARIABLES IN MALIGNANT MELANOMA: A 10 YEAR FOLLOW-UP STUDY. Mathew Rowley, and Clay J. Cockerell. Depts. of Dermatology and Pathology, Division of Dermatopathology, U.T. Southwestern Med Ctr., Dallas, TX.

Certain features of malignant melanoma have been shown to be indicators of prognosis both collectively and individually. Because long term survival occasionally is seen in individuals with multiple poor prognostic factors, we sought to evaluate the predictive value of age, sex, anatomic location, inflammatory infiltrate, mitotic index, thickness, vertical growth phase and presence of regression on prognosis of malignant melanoma. Histologic sections from 221 patients with malignant melanoma excised between the years 1977 and 1980 were evaluated for these features. In situ lesions and those with inadequate biopsy were excluded. Reliable 10 year follow-up data was obtained in 53. Seventy percent of patients survived for ≥10 years. Of these, 27% had a thickness ≥1.7mm with vertical growth phase present. Of long term survivors with lesions ≥1.7mm in thickness, 80% had 4 or more features associated with a poor prognosis. The overall predictive value of mortality when all variables were considered was 85%. Although the predictive value of these prognostic variables is generally reliable, there is significant population of long term survivors in which prognosis could not be accurately predicted using these features.

ROLE OF MELANIN PIGMENT IN THE MALIGNANT TRANSFORMATION OF MELANOCYTES T.G. Salopek, K. Jimbow. Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Alberta, Canada. In mammalian skin and hair, two types of melanin pigment may exist, either eumelanin or pheomelanin. These pigments vary dramatically in their ability to act as photoprotectors. Eumelanin appears to be an inert or protective substance against the detrimental effects of ultraviolet radiation (UVR). Pheomelanin, in contrast, has been shown to be photochemically unstable when exposed to UVR, generating free radicals, and resulting in impaired cell growth, cell lysis and increased mutagenicity. To assess the role of melanin pigment in the malignant transformation of melanocytes to melanoma, a cell-free system consisting of naked DNA (ccc pM2) and synthetic pigment (melanin free acid eumelanin and pheomelanin) were irradiated with UV-B light (λ = 290 and 310 nm). The DNA was assayed for single-strand breaks and the induction of apurinic sites using an Ethidium Bromide Fluorescence assay. Exposure of DNA to 310 nm light, in the presence or absence of pigment failed to induce any single-strand breaks or apurinic sites. Irradiation at 290 nm light produced a significant amount of single-strand breaks and a moderate amount of apurinic sites. The degree of single-strand induction or apurinic generation in the presence of pheomelanin or eumelanin at various concentrations was not statistically different from the controls. The present results question the role of the end-product of pheomelanin synthesis as a direct mutagen/carcinogen. It is postulated that pheomelanin may produce its in vitro cytotoxic effects via its precursors (i.e. 5-S-CD) by creating an imbalance between the free-radical producing systems and the protective cellular mechanisms.

ISOLATION OF cDNA CLONES FOR CANDIDATE MELANOMA SUPPRESSOR GENES. Saumyendra N. Sarkar, Aaron B. Lerner and Ruth Halaban, Department of Dermatology, Yale University School of Medicine, New Haven, CONNECTICUT,06510, U.S.A.

Frequent deletions or loss of heterozygosity at chromosomal location 1p in human melanomas suggest that this region plays a role in suppressing oncogenic events in melanocytes. We set out to isolate cDNA clones that are expressed in normal but not in malignant melanocytes in order to recover genes whose absence may contribute to malignant transformation and hence function as melanoma suppressor genes. We developed a new and efficient subtractive hybridization method to isolate such cDNA clones. Fourty four clones were isolated after two cycles of subtractive hybridization using normal melanocyte cDNA and mRNA from amelanotic metastatic melanoma cell lines with 1p deletions. Dot blot hybridization showed that 12 of these clones were expressed in normal melanocytes but not in melanoma cell lines. Northern blot analysis done on one of these clones (size approximately 500 b.p) confirmed this restriction of expression. The size of the hybridizing mRNA was approximately 1.4 kb. This cDNA clone was sequenced and no homology was found to other known genes when compared against a GenBank data base. The results suggest that we isolated a novel melanocyte-specific clone that is not expressed in metastatic melanoma cell lines with deletions at location 1p. Deletion of this gene or mutations that inactivate its function may be involved in the progression to melanomas. Alternatively, this clone may belong to a family of genes expressed in highly differentiated melanotic melanocytes known to become down-regulated in amelanotic melanomas.

ROLES OF Ca, cAMP, AND KINASE C FOR STIMULUS SECRETION COUPLING IN THE ECCRINE SWEAT GLAND. K. Sato, M. Ohtsuyama, K.T. Sato, G. Samman, and F. Sato. Marshall Dermatology Lab., Univ. of Iowa College of Medicine, Iowa City, IA.

Basic mechanisms of signal transduction such as receptor functions, Ca and cAMP cascades, phospholipid metabolism, and protein kinases are the universal cellular processes involved in regulation of a variety of cells including cutaneous appendageal cells. Eccrine sweat secretion is a consequence of complex intracellular and membrane processes including the increase in intracellular [Ca], K efflux from the cell due to activation of ion channels, cell shrinkage, and activation of transporters. Although the critical role of intracellular Ca in cholinergic sweat secretion is undisputable, it is not clear whether [Ca] alone suffices for cholinergic (MCH, at 3uM) signal transduction or whether other processes such as cAMP- or kinase C-mediated mechanisms are also involved. Sweat secretion and K efflux were determined by microcanulation and superfusion of isolated rhesus secretory coils, respectively, and cell volume and [Ca] determined by image analysis and fura 2 method, respectively, using collagenase dissociated clear cells. 1 mM ATP (sympathetic cotransmitter) increased cellular [Ca] to the level achieved by MCH, i.e. from 100-200 nM at rest to 500 to 900 nM after stimulation). Nevertheless, the ATP-stimulated K efflux, sweat rate, and cell shrinkage were only 12%, 5%, and 50% those of MCH, suggesting that Ca alone is insufficient for the maximal signal transduction. ATP-stimulated K efflux was enhanced 48% by 5 mM theophylline, 30% by 50uM forskolin, and 32% by 1uM phorbor ester (TPA, stimulant of kinase C). 10uM ionomycin (IM), which elevates [Ca] to >4uM, induced K efflux and cell shrinking only half as much as did MCH but the IM-induced K efflux was stimulated 140% by TPA. The data indicates that cAMP- and/or protein kinase C augment the effect of intracellular Ca in stimulus secretion coupling and that all of these processes' are physiologically involved in regulation of glandular function.

THE EFFECT OF MITOGENS AND ANTI-MITOGEN ON HUMAN KERATINOCYTE LOCOMOTION: EVIDENCE FOR INDEPENDENT MECHANISMS OF CELL LOCOMOTION AND PROLIFERATION. Yves Sarret, Kimberly Grigsby, David T. Woodley, Univ of North Carolina, Chapel Hill, NC and Stanford Univ, Stanford, CA.

Using a newly-described human keratinocyte (HK) locomotion assay, we examined the effects of the known mitogens - epidermal growth factor (EGF), fibroblast growth factor (FGF) and bovine pituitary extract (BPE) - and an anti-mitogen, transforming growth factor-Beta (TGF-B), on HK locomotion. HKs cultured in complete MCDB 153 medium (with EGF and BPE) were suspended and placed in petri dishes on coverslips coated with gold salts alone or with the addition of adsorbed collagens I and IV. Cells were then cultured in MCDB medium with or without EGF, FGF, BPE or TGF-B and stopped 10 hours before the doubling time with formaldehyde. Multiple, random, non-overlapping fields of the dishes were photographed and cell migration quantitated by computer-assisted image analysis that expresses locomotion as the percentage of the total field area displaced by the area of cell tracks through the gold salts (Migration Index = MI). In complete medium, MIs on plastic, type I and type IV collagen were 2%, 30% and 35%, respectively. TGF-B (1to10ngs/ml) inhibited HK tritiated thymidine incorporation 80-95% but had no inhibitory or promotion effect on HK locomotion. Assays performed in MCDB medium depleted of EGF and BPE demonstrated a 50% decrease in migration compared with complete medium. The addition of EGF, TGF-B or FGF to depleted MCDB medium did not restore HK locomotion, but the addition of BPE enhanced HK locomotion between 15-20%. These data show (i) that total inhibition of HK proliferative potential does not alter collagen-driven HK locomotion, (ii) that EGF and FGF, potent HK mitogens, neither enhance or inhibit HK migration and (iii) that a component of BPE promotes HK locomotion on collagen. The data support the concept that HK locomotion and proliferation operate by independent cellular mechanisms.

AUTOCRINE STIMULATION OF KERATINOCYTES BY INTERLEUKIN 3 (IL-3): MURINE KERATINOCYTES POSSESS IL-3 RECEPTORS. D.N. Sauder[1], S. Matic[1], D. Fong[2] and V. Duronio[2], [1]Dept. of Medicine, McMaster University, Hamilton, Ont., and [2]Biomedical Research Centre, Vancouver, B.C., CANADA

The murine keratinocytes cell line PAM 212 has previously been shown to synthesize and secrete IL-3. Studies were undertaken to determine if PAM cells respond to IL-3 and if IL-3 receptors were present in murine keratinocytes. IL-3 was chemically synthesized by means of an automated peptide synthesizer (applied Biosystem 430A) as previously described. IL-3 stimulated a dose dependent proliferation of keratinocytes showing increased cell number from $8.8x10^6$ in untreated cultures to $14.6x10^6$ cells for IL-3 treated cultures. IL-3 also increased thymidine incorporation from 10,056 ± 547 for untreated to 38,721 ± 6,100 for IL-3 treated. Maximum stimulation was seen in 1ng/ml. In addition to IL-3 induced proliferation, IL-3 also caused a dose dependent increase in keratinocyte migration using a modified Boyden chamber assay. Peak migration was detected at 5ng/ml. These results suggested that PAM cells possess IL-3 receptors. Radioligand binding studies confirmed the presence of IL-3 receptors on PAM cells. Keratinocytes in log phase growth express more receptors than quiescent cells, with the former having approximately 1000 receptors per cell. These studies reveal that the murine keratinocyte cell line and PAM 212 possess IL-3 receptors and respond to IL-3 by proliferation and directed migration.

INTERLEUKIN I IN HUMAN ECCRINE SWEAT. Fusako Sato and Kenzo Sato. Marshall Dermatology Research Laboratories, Department of Dermatology, University of Iowa College of Medicine, Iowa City, IA.

Interleukin 1 (IL-1), a hormone-like polypeptide, is produced by a wide variety of cells including keratinocytes, fibroblasts, and lymphocytes and mediates inflammation, proliferation, differentiation, prostaglandin metabolism, and signal transduction. Although human eccrine sweat has been reported to contain IL-1, the detailed mechanism of its secretion remains to be studied. Using radioimmunoassay, we detected [IL-1α] in thermally induced human eccrine sweat collected over a vaseline barrier in a airtight plastic film to minimize evaporation and contamination (clean sweat). When pooled sweat was ultrafiltrated (YM10 membrane) and fractionated by a Superose FPLC column (PBS 150 mM at pH 7.1), a single peak IL-1α activity was found at 17 KD. [IL-1α] in scraped sweat was 2-5 x higher than that in clean sweat, indicating that significant epidermal contamination can occur if adequate precaution is not taken. Significant individual difference in [IL-1α] was observed in clean sweat; 120-700 pg/ml sweat in men and 350-2400 pg/ml in women (both n=6,ages 18-26) which is not correlated with the individual difference in sweat $rate_{max}$ nor with serum [IL-1α] (a mean of 87 pg/ml), present in all the sweat samples regardless of sequence of sample collection, but tends to slightly increase with increasing sweat rate. [IL-1β] was lower than 100 pg/ml. [IL-1α] in the axillary sweat was 2-6 x higher. IL-1α was also demonstrable in the supernatant of primary cultured human secretory coil cells and the ductal cells grown on a permeable support at 5-35 pg/cm² of monolayer per day, which was nearly doubled in the presence of 10^{-5}M methacholine (n=2) or isoproterenol + 1 mM theophylline (n=2). We conclude that biologically significant amounts of IL-1α are produced by the the sweat gland and are present in human eccrine sweat.

ELEVATED UNOCCUPIED GLUCOCORTICOID RECEPTORS IN SCALP OF PATIENTS WITH ALOPECIA AREATA. Marty E. Sawaya, Maria K. Hordinsky*, Robert J. Cohen, Keith A. Harris, and Lawrence A. Schachner. University of Miami School of Medicine, Miami, FL, *University of Minnesota Health Center, Minnepolis, MN

Alopecia areata (AA) patients show a variable response to local, short term glucocorticoid (GC) treatment regimens. Exposure to potent GC implies a "switch" mechanism in AA, as seen with other diseases with an "all or none" response. Our investigations seek to understand this response, by analyzing glucocorticoid receptor (GCR) content in human scalp of normal (N), AA, and androgenetic alopecia (AGA) patients. Fifteen patients with AA, with no prior GC treatment were biopsied. The epidermis and dermis were separated from subcutaneous fat and analyzed for Total and Unoccupied GCR type I & II binding in cytosol and nuclear fractions, by the dextran coated charcoal method. Results indicate approximately two fold greater type II unoccupied GCR in the cytosol and nuclear fractions in AA, versus N and AGA scalp tissue. Type II GCR has been shown in other tissues to influence long term, slow growth cellular processes. Unoccupied GCR from AA was also assessed for GCR-binding activation. Two components in cytosol are required for this GCR activation: NADPH and a thioredoxin-mediating reducing system, called thioredoxin reductase (TR). Our results show decreased TR levels in AA when compared with N or AGA scalp. Overall, elevated unoccupied type II GCR, with diminished TR levels (which facilitates and activates GC binding to the GCR), may be important in explaining the variable responses seen with GC treatment for hair regrowth in patients with AA.

ELEVATED TYPE II GLUCOCORTICOID RECEPTOR BINDING IN KELOIDS & HYPERTROPHIC SCARS. Marty E. Sawaya, Robert S. Kirsner, Albert J. Nemeth, Darryl S. Weiss, and S.L. Hsia. University of Miami School of Medicine , Miami, FL

Keloids are benign dermal tumors resulting from an abnormal wound healing process in genetically predisposed individuals. Kenalog, a potent glucocorticoid (GC) is often injected at the keloid site to retard or inhibit growth by suppressing collagen production and cell proliferation. The biochemical mechanisms in which GC's mediate these processes have not been fully elucidated, and are the focus of our work. Previous studies showed no differences in the "classical" glucocorticoid receptor (GCR) type I binding in keloids vs. normal fibroblasts. In the present study, keloids and hypertrophic scars from 17 patients were excised, homogenized, and assessed for GCR type I & II binding by the dextran coated charcoal method. Results for type I binding were marginally higher for keloid than normal tissue, however, for type II nuclear GCR binding there was approx. 2.2 times greater binding capacity (342±67 fmole/mg DNA) in keloids versus normal tissue (152±46 fmole/mg DNA). As well, "activation" capacity for these elevated type II receptors was tested and greatly differed in keloid versus the normal tissue. Nuclear type II binding has been found in other tissues, such as uterus, to affect long term, slow growth cellular processes. Type II GCR binding may be induced by local GC treatment, as with Kenalog, and may play a role in suppressing keloid growth processes, as our present work is showing.

KARYOTYPIC VARIATIONS IN TUBEROUS SCLEROSIS.
M.A. Scappaticci*, C. Danesino*, F. Cottoni°, S. Rubino** and G. Orecchia*. Dipartimento di Patologia Umana ed Ereditaria, Pavia*; Clinica Dermatologica, Sassari°; Istituto di Microbiologia**, Sassari. Italy.

We have performed cytogenetic investigation on cultured fibroblasts derived from biopsies of a "café au lait" spot a hypopigmented spot, a sebaceous adenoma and a shagreen patch, from 8 cases of tuberous sclerosis (TS). The chromosomes were analyzed at the 2nd passage. We have found karyotypic variations in alla cases: presence of metaphases with a premature centromere disjunction (PCD) of the chromosomes, micronuclei, increase a polyploid cells and breaks. Moreover we studied the organization of some cytoskeleton proteins of TS cell line with monoclonal antibodies to tubuline and vimentine and with rodamine conjugated phallotoxin, which binds F-actine, but we did not evidence anomalies. In conclusion our findings suggest that karyotypic variations can be considered a cellular phenotypic characteristic of TS in fibroblasts cultured from the skin lesions. The PCD phenomenon, the presence of micronuclei and polyploid cells indicate a possible disturbance in the mechanisms of centromere division and in the chromosome distribution at cell division.

AGE RELATED MOLECULAR PROTEINS AFFECTING HAIR GROWTH IN MEN WITH ANDROGENETIC ALOPECIA. Marty E. Sawaya, Craig A. Kraffert, Leonard A. Lewis, Manuel Iriondo and S.L. Hsia. University of Miami School of Medicine, Miami, FL

Is there a "point of no return" to stimulating hair growth in men with androgenetic alopecia (AGA) ? Target and non-target tissue differ in androgen sensitivity because non-target tissue nuclear chromatin possesses acidic proteins that "mask" the chromosomal-protein androgen-receptor (AR) acceptor sites. The number of available acceptor sites in target tissue changes during development or in age of the organism because of such masking, the consequence of which is to alter gene expression. Chromatin isolated from hair follicle (HF) of young (20-45 yr), and mature (52-70 yr) men with AGA was assessed for saturation binding of purified ^{3}H-labelled AR complexes. Saturation binding was measured after extraction of histones and masking acidic proteins. Binding was greater in young men (n=6), suggesting the presence of a greater number of AR acceptor sites. The low binding seen in the mature group, resulted from the greater content of masking proteins. Binding increased after removal of these histones and masking-acidic proteins. Various intermediary components and events in the androgenic signal transduction sequence were shown to deteriorate > 40% in the "mature" group. These include total nuclear AR concentration, and chromatin binding of AR complexes, and stimulation of nuclear RNA polymerase II, thus, altering gene expression. These results give an indication that there may be a "point of no return" to stimulating hair growth during the aging process.

A COMPARATIVE STUDY OF THE THIOPROTEINS THIOREDOXIN REDUCTASE AND GLUTATHIONE REDUCTASE IN HUMAN KERATINOCYTES.
Karin U. Schallreuter[1], Mark R. Pittelkow[2] and John M. Wood[3].
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The aim of this study was to determine the influence of thioredoxin reductase (TR) and glutathione reductase (GR) on the intra-cellular redox-status of human keratinocytes established from normal healthy donors, from patients with tyrosinase positive albinism (HPS) and vitiligo. Keratinocytes were grown in synthetic medium containg $0,1 \times 10^{-3}$M calcium. TR and GR were purified by FPLC on an HR 5/5 Mono Q column using a NaCl gradient. TR eluted at 0.12M and GR at 0.16M NaCl. TR and GR activities were assayed by DTNB reduction at 412nm. Intra-cellular reduced thioredoxin (T red) was determined by incubating cells with ^{14}C methylmercurichloride (52mCi/mMole) followed by separation of the total thioredoxin with FPLC at 0.3M NaCl. Intra-cellular pH for keratinocytes was measured from the chemical shift of cytosol orthophosphate using ^{31}P NMR. In human keratinocytes the TR/T system represents 5% of the total acidic cytosol protein fraction, whereas GR was barely detectable. ^{31}P NMR revealed an in-vivo cytosol pH 7,05. Since the pK$_a$ for the TR thiol active site is 6,98, then the pH may cause the induction of TR over GR in the epidermis. Keratinocytes established from patients with HPS, vitiligo and controls (skin types II a. IV) revealed intra-cellular T red with HPS > vitiligo > skin type II > skin type IV. The data underline the importance of the redox conditions of keratinocytes for regulation of pigmentation in the human epidermis.

HUMAN HAIR FOLLICLE AROMATASE ACTIVITY IN FEMALES WITH ANDROGENETIC ALOPECIA. Marty E. Sawaya, Vera H. Price*, Keith A. Harris, Robert S. Kirsner, and S.L. Hsia. University of Miami School of Medicine, Miami, FL, *University of California San Francisco, San Francisco, CA.

The degree of hair loss in women with androgenetic alopecia (AGA) is much less than in men. Most women with diffuse alopecia experience a gradual loss of hair on the central scalp, with retention of the frontal hairline. Although it has been assumed that the hormonal basis for AGA in women is the same in men, no studies have been done to confirm this. Six women, ages 18-30 with AGA were selected for 4 mm biopsy from the balding, frontal (B) and hairy, occipital (H) scalp sites. We describe studies of the enzymes mediating androgen action: Δ^5-3B-hydroxysteroid dehydrogenase (3B-HSD), 17B-hydroxysteroid dehydrogenase (17B-HSD), 5a-reductase (5aR), and aromatase (A) enzyme in the hair follicles. Apparent Km values did not differ for each enzyme whether present in B or H. However, levels of **specific activity** varied greatly, especially in comparison to males with AGA. Remarkable differences in conversion of 4-androstenedione, and testosterone into aromatized metabolites such as estrone and 17B-estradiol was seen in female specimens, with the H sites having greater levels than the B sites from 5 out of 6 women. In men with AGA, negligible levels of aromatase was detected. Aromatase has been described in many other organ systems, including skin and fat. This enzyme may be important for understanding biochemical differences for AGA in women, where sparing of the frontal hairline may be due to the decreased levels of potent androgens effecting hair follicles due to local conversion to estrogens.

IgG AUTOANTIBODIES TO IL-1 ALPHA IN THE SERUM OF PATIENTS WITH SKIN DISEASES. INCIDENCE, CHARACTERIZATION AND EFFECT ON THE BLOOD CLEARANCE OF IL-1 ALPHA. Didierjean L, Schifferli J*, Steiger G* and Saurat JH.
Departments of Dermatology and *Medicine, University Hospital, Geneva, Switzerland.

Autoantibodies to cytokines may play a role in the regulation of the cytokine network in vivo. Since we previously identified autoantibodies to IL-1 alpha in the serum from a patient with the syndrome of Urticaria, Fever and Macroglobulinemia (UFMs) (JID 1990, in press), the present study was designed to further analyse this phenomenon.

IL-1 alpha binding factor (BF) was identified by PEG precipitation of iodinated IL-1 alpha in 7/11 (64%) of patients with UFMs and in 29/167 of controls (17%) ($p = 0.003$); density-gradient centrifugation for IL-1 alpha BF gave similar results. The binding activity was specifically abolished by an excess of unlabeled IL-1 alpha but not by IL-1 ß or TNF, and was not found to be related to uromodulin and ß2 M. IL-1alpha BF was identified as IgG because : (i) no IgM binding activity could be identified, (ii) analysis by Ouchterlony immunodiffusion of the gradient-density centrifugation peak showed that IL-1 was precipitated with anti IgG indicating the presence of IL-1-IgG complex, (iii) ligand blotting was found positive at the position of IgG, (iv) protein G IgG depletion abolished IL-1 alpha binding activity and (v) purified IgG from positive sera and their F(ab)-2 fragments showed binding activity. As compared to IL-1 alpha BF negative IgG, the injection in rats of IL-1 alpha BF positive IgG increased four fold the blood half life of exogenous iodinated IL-1 alpha and decreased its diffusion in tissues like muscle and skin.

The data indicate that (i) the incidence of IgG autoantibodies to IL-1 alpha is about 15% in normal sera and is not elevated in common inflammatory dermatoses except the rare UFMs; (ii) anti IL-1 alpha IgG interferes with the clearance of blood IL-1 alpha, an observation that might be of biological significance.

THE EFFECT OF STRUCTURAL VARIATIONS OF ENHANCERS ON SANDIMMUNE-PENETRATION IN THE IN VITRO RAT SKIN MODEL. Fritz Schmook, Anton Stütz, and Fritz Richter, Sandoz Forschungsinstitut, Vienna, Austria, and Berne, Switzerland.

The enhancement of Sandimmune (Cyclosporine A, SIM) penetration by various enhancers was tested in an in vitro rat skin model in Franz-type diffusion cells with skin of hairless rats.

The chain length, number and position of double bonds, branching of the carbon chain of the enhancer as well as its concentration and the influence of the solvent was investigated. SIM was determined by HPLC.

The penetration rates decrease with increasing number of double bonds of the enhancer and decreasing SIM- and enhancer concentration in the donor solution, but increase with increasing chain length of the enhancer molecule up to a maximum at 18 to 22 carbon atoms. Enhancers increase penetration rates by a factor of 20-50 for alcoholic and maximally 5 for oily formulations.

Skin concentrations are maximal at SIM-concentrations of ca. 5 % in alcoholic formulations, but are proportional to SIM-concentrations in oily test compositions. For formulations containing triglycerides skin concentrations increase with decreasing chain length of the fatty acids. Enhancers increase skin concentrations by a factor of 10-25 for alcoholic and 4-20 in oily formulations.

Structural parameters of the enhancer and solvent used considerably influence the penetration of SIM into and through rat skin. Thus, the composition of galenical formulations may play a critical role for clinical efficacy of topical SIM.

ALTERATIONS IN COLLAGEN IN HUMAN SUN-DAMAGED SKIN. E. Schwartz, J.S. Perlish, F.A. Cruickshank, and R. Fleischmajer, Department of Dermatology, Mt. Sinai School of Medicine, New York, N.Y.

Previous work has demonstrated that pNα_1(III) procollagen is lost from the surface of collagen fibers in UV irradiated mouse skin. This alteration may allow collagen to interact with elastin to form the elastotic material. The aims of this study were to quantify pNα_1(III) procollagen in sun-damaged human skin (cutis rhomboidalis nuchae) and to demonstrate the interaction of elastin with collagen in the elastotic material. Extracts of sun-damaged and nonsolar exposed (control) skins were subjected to immunoblotting techniques following SDS-PAGE with 5.5% gels using antibodies directed against the aminopropeptide of type III procollagen. The percentage of pNα_1(III) procollagen in the extracts (calculated by scanning densiometry) decreased significantly ($p < 0.01$) from 29.5% (± 2.8 SEM, n=3) in the control skins to 8.6% (± 2.9 SEM, n=3) in the sun-damaged samples. Small pieces of sun-damaged skin were treated with elastase and then incubated with antibodies directed against elastin and types I and III collagens for immunofluorescence studies. The elastotic material of untreated skin reacted intensely with elastin antibodies but not with the collagen antibodies. However, collagen fibers were visualized in the elastotic material of elastase-treated skin. Reactivity with elastin antibodies was removed by elastase treatment. These results indicate that pNα_1(III) procollagen is lost in human sun-damaged skin and that the elastotic material consists of elastin and collagen fibers.

CUTANEOUS IMMUNOPATHOLOGY OF ALCOHOLIC LIVER DISEASE. Arnold Schrocter, Mohammad Saklayen*, Mary Ann Nafz, Department of Dermatology, Wright State University, School of Medicine, Dayton, Ohio. *Veterans Administration Medical Center, Dayton, Ohio.

Alcoholic injury to liver and intestinal mucosa may induce excessive production of IgA circulating antibodies and IgA antibody deposits in vessels of the liver, jejunum, and skin (Kater, et al., 1979, Swerdlow, et al., 1983). The sensitivity and specificity of IgA deposits in the skin of patients with alcoholic liver disease has not been completely determined. We have investigated the cutaneous immunopathologic findings of alcoholic liver disease by direct immunofluorescence on skin biopsies from uninvolved, unexposed areas. The biopsies were taken from 18 patients with known alcoholic abuse, most (16) with liver cirrhosis. The primary antibody found was IgA in a granular pattern on the basement membrane of the eccrine secretory coils and/or in vessels around this same structure or in the papillary dermis. Eighty-three percent of the biopsies tested had this feature. All cutaneous biopsies from alcoholic liver disease patients had C3 in the dermal vessels. On the other hand, biopsies from patients with dermatitis herpetiformis (3), which have dense, granular deposits of IgA antibody at the dermal/epidermal junction, had little or no granular IgA deposits at the basement membrane of the eccrine secretory coils or in the vessels. Random testing of specimens from subjects with a history of little or no alcoholic intake (24) showed no deposits of IgA antibodies. These included a hepatitis B patient with resulting cirrhosis, but no alcoholic intake. All of these results indicate that there is a direct correlation between excessive alcoholic intake, alcoholic liver disease and cutaneous deposits of IgA antibodies. IgA skin deposits could provide a less invasive method for assessing immediate or prolonged alcoholic intake, as well as resulting liver damage.

METHOTREXATE INDUCES DIFFERENTIATION IN HUMAN KERATINOCYTES IN VITRO. P.M. Schwartz, S.K. Barnett, E.J. Atillasoy and L.M. Milstone, Department of Dermatology, VA Medical Center, West Haven, CT and Yale University, New Haven, CT.

Methotrexate (MTX) inhibits epidermal proliferation in vivo and the growth of human keratinocytes in vitro. This growth-inhibitory effect of MTX is reversed by thymidine. We report here that MTX induces differentiation in human keratinocytes and that this action of MTX is also prevented by exogenous thymidine. MTX causes a marked change in cell morphology within 24-48 hrs. In human keratinocytes growing in complete MCDB 153 lacking thymidine, 0.1uM MTX for 48 hrs inhibited growth by 50%, increased the average cell volume from 4710um^3 to 15300um^3 and increased the proportion of involucrin positive cells from 20% to >75%. The effect of MTX on cell proliferation, morphology and expression of involucrin was reversed by the addition of thymidine to the culture medium. Thymidine (5uM) added simultaneously or 6 hrs after treatment of cells with concentrations of MTX as high as 20uM negated the effect of the drug. MTX may therefore be a useful tool to study the mechanistic link between inhibition of DNA synthesis caused by a depletion of thymine deoxynucleotides and induction of differentiation. These data suggest that the capacity of MTX to induce differentiation may be relevant to its therapeutic action in psoriasis.

HEXAMETHONIUM SUPPRESSES THE NICOTINE-PROVOKED INCREASE IN CUTANEOUS ERYTHROCYTE FLUX. Linda Schulski and Jonathan K. Wilkin, Departments of Medicine (Dermatology) and Pharmacology, Ohio State University College of Medicine, Columbus, Ohio

Topically applied nicotine is a cutaneous vasodilator demonstrating tolerance and relative stereoselectivity. To examine if antagonism to the nicotine receptor would block this cutaneous vascular response, 1% hexamethonium bromide (C_6) or vehicle alone was applied to the volar forearm surface of 10 nonsmoking men. The stratum corneum was removed at pretreatment sites before pharmacologic agents were applied. Nicotine base in 0%, .01%, 0.1%, and 1% aqueous solution was subsequently applied to treatment sites. Dose-erythrocyte flux response curves were obtained using laser Doppler velocimetry. C_6 pretreatment led to a significant decrease in erythrocyte flux (mean ± SEM) response at all nicotine concentrations ($p < .001$):

Nicotine	0.0%	.01%	0.1%	1.0%
C_6	47±7	77±13	101±22	212±26
vehicle	56±8	206±26	288±38	314±39

To exclude a nonspecific vasoconstrictor effectof C_6, erythrocyte flux in 5 subjects pretreated at different sites with C_6 and vehicle as above was measured using a heated laser Doppler probe at 23°C and 41°C. No differences in erythrocyte flux were demonstrated between controls and C_6 treated areas. These results imply that nicotine augments cutaneous erythrocyte flux via nicotine receptors, since this response can be specifically antagonized.

LOCALIZATION OF BASIC FIBROBLAST GROWTH FACTOR mRNA IN MELANOCYTIC LESIONS BY *IN SITU* HYBRIDIZATION. G Scott, M Stoler, S Sarkar, R Halaban. Dermatology and Pathology Depts, Univ of Rochester, Rochester, NY, Dermatology, Yale Univ, New Haven, CT, USA.

Basic fibroblast growth factor (bFGF) is a potent mitogen for normal human melanocytes and keratinocytes in culture. Experiments *in vitro* suggest that keratinocytes supply bFGF to melanocytes through a paracrine mechanism and that the aberrant expression of bFGF in melanomas confers growth independence from bFGF-producing cells. To determine whether bFGF is expressed *in vivo*, we examined a series of benign and malignant melanocytic lesions *in situ* using bFGF riboprobes on tissue sections, and correlated bFGF expression with histologic phenotype. Seventeen melanocytic neoplasms were studied, including 4 common acquired nevi, 4 dysplastic nevi, 4 primary malignant melanomas and 5 metastatic melanomas. Nevic cells in benign intradermal nevi showed low signal intensity (1+) while compound and dysplastic nevi showed 2+-3+ expression in the junctional nevic cell population and 1+ expression in the dermal nevic cell population. Melanocytes in primary melanomas had intermediate (2+) and those in metastatic melanomas low (1+) levels of bFGF gene transcripts. Fibroblasts and epidermal and adnexal keratinocytes expressed high levels (3+) of bFGF in all the cases studied. Basic FGF expression in endothelial cells, known to produce and respond to this growth factor *in vitro*, was lower than that in fibroblasts, keratinocytes and the nevomelanocytic cell population, and in 10 out of 17 cases, no bFGF mRNA was detectable. This study shows that bFGF is expressed in nevomelanocytes *in vivo* in all melanocytic lesions and thus cannot be used as a marker for transformation. The presence of bFGF gene transcripts in the various dermal cell types suggests that it is an autocrine growth factor that may regulate cellular proliferation in the skin.

EXPRESSION OF CELL-CELL ADHESION MOLECULES IN MELANOCYTE SIGNALING IN THE WHITE LEGHORN CHICK EMBRYO: AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY. G. Searles, K. Jimbow. Div. Derm. & Cut. Sci., U. of Alberta, Edmonton, AB

The mechanism of contact communication between melanocytes (MC) and keratinocytes (KC) in normal and vitiliginous skin is unknown. We selected cell surface adhesion molecules (CAM) as a tool to study White Leghorn Chick cytotoxic vitiligo model, which has a predictable programmed cell death in embryo, and loss of melanosome (MS) transfer as the first event. In order to see the role of CAM in MC signaling in hypomelanotic states, we examined (a) whether CAM is expressed on MC; (b) if CAM expression is altered during dysfunctional MS transfer; (c) whether CAM is a signal molecule controlling MS transfer. We treated Day 7-20 chick embryos with anti-neural CAM and anti-liver CAM antibodies using ABC and immunogold immunohistologic methods. Our results show that (a) N-CAM has 2-3x higher expression on early MC than KC; (b) expression decreases on Day 8, and is lost by Day 10; (c) free MS mimic MC N-CAM expression; (d) loss of CAM expression preceeds loss of MC transfer by one day; (e) L-CAM is only expressed on KC and does not change with MC dysfunction. This first report of CAM expression on developing chick MC stresses the importance of (a) N-CAM as primary CAM on MC; (b) change in N-CAM expression occurs prior to detection of the earliest marker of MC dysfunction; (c) abnormal CAM expression may cause dysfunctional MS transfer signaling and function seen in early stages of acquired hypomelanotic states like alopecia areata and vitiligo.

GAMMA-INTERFERON SELECTIVELY SUPPRESSES BASAL AND LPS-INDUCED METALLOPROTEINASE PRODUCTION BY HUMAN MACROPHAGES. Steven D. Shapiro, Edward J. Campbell, and Howard G. Welgus, Divisions of Dermatology and Respiratory and Critical Care, Jewish Hospital at Washington University Medical Center, St. Louis, Missouri.

Gamma-interferon, a lymphokine capable of activating macrophages, has been found to selectively diminish the production of three metalloproteinases by the mononuclear phagocyte: interstitial collagenase, 92 kD type IV collagenase, and stromelysin. Inhibition of constitutive and LPS-induced metalloproteinase expression occurred at a pretranslational level, as evidenced by reductions in enzyme biosynthesis and collagenase-specific steady-state mRNA levels. Metalloproteinase production was quite sensitive to the cytokine, diminishing by >50% at doses of gamma-interferon of 10-40 Units/ml. These and even higher concentrations of the cytokine actually enhanced general protein synthesis, indicating a highly specific suppressive effect on metalloenzyme biosynthesis. The secretion of the counter-regulatory tissue inhibitor of metalloproteinases (TIMP) was also decreased by gamma-interferon, but doses 10 to 25-fold higher were required than those producing metalloenzyme shutdown. Interestingly, metalloproteinase and TIMP synthesis by human skin fibroblasts were refractory to the action of gamma-interferon, despite the presence of receptors on such cells and a decrease in the synthesis of collagen in response to the cytokine. Therefore, while gamma-interferon activates the macrophage into a tumoricidal cell, such biologic function appears to be attained at the expense of the cell's capacity to degrade extracellular matrix.

MUTATIONAL HOTSPOT VARIABILITY IN AN ULTRAVIOLET-TREATED SHUTTLE VECTOR PLASMID PROPAGATED IN XERODERMA PIGMENTOSUM AND NORMAL HUMAN LYMPHOBLASTS AND FIBROBLASTS Saraswathy Seetharam, Kenneth H. Kraemer, Haywood L. Waters, and Michael M. Seidman, National Cancer Institute, Bethesda, MD, and Otsuka America Pharmaceutical,Inc., Rockville, MD

The mutagenesis shuttle vector, pZ189, was treated with ultraviolet radiation (UV) in vitro and passed through a DNA repair deficient lymphoblastoid cell line derived from a patient with xeroderma pigmentosum complementation group A (XP-A) (XP12BE(EBV)) and a DNA repair proficient lymphoblastoid cell line (GM606(EBV)). After UV treatment, plasmid survival was lower and mutation frequency higher with the XP-A cells mirroring the survival and mutagenesis of the host cells. The nature of the mutations in the suppressor tRNA marker gene was determined by direct sequence analysis. The G:C to A:T transition was the dominant (85%) base substitution mutation with the XP lymphoblasts and was the major (56%) base substitution mutation with the repair proficient lymphoblasts. We found a G:C to A:T transition mutational hotspot with the XP lymphoblasts not seen in our previous experiments with fibroblasts from the same patient (Proc Natl Acad Sci 83, 8273-7, 1986). Comparison of the data presented here with our results with DNA repair deficient and DNA repair proficient fibroblasts suggests that hotspot variability is not due to genetic polymorphism or repair capacity of the cells. Instead it appears that cellular factors can influence the probability of mutagenesis of modified DNA at particular sites.

INHIBITION OF CONTACT HYPERSENSITIVITY AND PMA-INDUCED INFLAMMATION WITH 8-[3-(p-FLUOROBENZOYL)PROPYL]-1-PHENYL-1,3,8-TRIAZASPIRO-[4.5]DECAN-4-ONE R.J. Sharpe, K.A. Arndt, Z. Wang and S.J. Galli, Beth Israel Hospital and Harvard Medical School, Departments of Dermatology and Pathology, Boston, MA.

8-[3-(p-Fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one, was given s.c. to mice sensitized 6 days earlier to the hapten oxazalone. If the agent was given (300 mg/kg, s.c.) 1 hr after challenging the mice via topical application of oxazalone to the ear, the cutaneous hypersensitivity response was almost completely abrogated (Δ ear thickness at 24 hrs, with vs without treatment = 4.7 vs 185×10^{-4} inches, $p < 0.01$) Similarly, if an immunologically non-specific acute inflammatory response was induced in mouse skin with phorbol-12-myristate-13-acetate (PMA) a similar result was obtained (Δear thickness at 18 hrs, with vs without treatment = 1.7 vs 167×10^{-4} inches, $p<0.01$). Histologic analysis indicated that, in addition to diminishing swelling associated with these reactions, this agent markedly reduced leukocyte infiltration into challenged sites. Moreover, the agent can reduce cutaneous inflammation when administered topically and much lower systemic doses are also effective. We suggest that this agent, and its analogues and derivatives, may be useful in treating immunologic and/or inflammatory conditions such as contact dermatitis, atopic eczema, psoriasis, allograft rejection and autoimmune diseases. For treatment of inflammation of the skin, eye and mucous membranes, topical application of these agents may limit their systemic toxicity, while providing effective local concentrations.

SPECIFIC SKIN MANIFESTATIONS IN ACUTE (MYELO) MONOCYTIC LEUKEMIA: A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF NINE CASES. N. Sepp[1], Th. Radaszkiewicz[3], C. Meijer[4], J. Smolle[2], P. Fritsch[1], H. Kerl[2].
Depts. of Dermatology, Univ. of Innsbruck[1] and Graz[2], Austria; Dept. of Pathology, Univ. of Vienna[3], Austria; Dept. of Pathology, Univ. of Amsterdam[4], The Netherlands.

Acute monocytic leukemia can be divided into two types: a myelomonocytic type and a monocytic type. Infiltration of the skin in acute monocytic leukemia occurs in 10-20% of patients. Occasionally, skin lesions are the first symptom, even preceding monocytosis. We have studied nine patients with myelomonocytic (n=1) and monocytic leukemia (n=8) clinically, histologically and by immunohistochemistry (paraffin embedded sections, frozen material - 3-step immunoperoxidase method). Clinically, all patients showed disseminated papules or tumours. Histologically, diffuse infiltrates were found and frequently arranged in a characteristic "whirl" pattern. In paraffin material (n=7), the cells revealed positivity for lysozyme, chloracetate-esterase (n=3), LCA (CD45), MT1 (CD43), LeuM1 (CD15) (n=4) and LN2 (CD74)(n=3). Weak staining was found with MB1 (CD45R), LN1 and Mac387. On frozen sections (n=5), the cells stained with Leu3a (CD4), Vim12 (CD16), VimD5 (CD15), Vim2(w65), KiM7 (CD68), My7 (CD13), anti-mono (p200) and My9 (CD33) in all 5; 4 of them stained with LeuM5 (CD11c) and KiM6 (CD69). An interesting finding was the CD4 expression on neoplastic cells. In this study we report for the first time a detailed immunohistological analysis of specific skin lesions in a large group of patients with acute (myelo) monocytic leukemia.

RECOMBINANT PLATELET FACTOR 4 (PF-4) RECRUITS NEUTROPHILS AND MONONUCLEAR CELLS *IN VIVO*. R.J. Sharpe, G.F. Murphy, D. Whitaker and T.E. Maione, Repligen Corporation, One Kendall Square, Cambridge, MA, Beth Israel Hospital and Harvard Medical School Department of Dermatology, Boston, MA, and University of Pennsylvania, Department of Dermatology, Philadelphia, PA.

PF-4, a protein with homology to NAP/IL-8 and beta-thromboglobulin, is chemotactic for neutrophils and mononuclear cells *in vitro*. We tested recombinant human PF-4 (rPF-4) for its ability to recruit these cells *in vivo*. Injection of 25 µg of rPF-4 into the murine dermis results in rapid footpad swelling which peaks at between 6 and 12 hours. Histologic examination at various time points after the single 25 µg injection of rPF-4 reveals the principal infiltrating cells to be neutrophils and to a lesser degree mononuclear cells with a striking perivascular distribution. Chronic injection of rPF-4 results in an almost equal number of neutrophils and mononuclear cells. Marked fibrosis is seen after only 5 days of daily injection of 25 µg of rPF-4 and this has potentially important implications with regard to sclerosing disorders such as scleroderma. The pro-inflammatory effect could be reproduced with a 41 amino acid PF-4 carboxy terminal peptide, but not the 29 amino acid amino terminal peptide. Local release of PF-4 by mast cells, basophils or aggregating platelets may act alone or in combination with other mediators such as IL-1 or TNF-alpha in contributing to host defense against infection and neoplasia as well as the pathogenesis of autoimmune disease.

INDUCTION OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN CULTURED ENDOTHELIUM BY RECOMBINANT PLATELET FACTOR 4 (PF-4), Richard J. Sharpe, Theodore E. Maione, Diana Whitaker, and George F. Murphy, Repligen Corporation, Cambridge, MA, Beth Israel Hospital and Harvard Medical School Department of Dermatology, Boston, MA, and University of Pennsylvania, Department of Dermatology, Philadelphia, PA.

PF-4 is a member of a family of gene products which includes the mediators of inflammation; MIP-1 and 2, IL-8 and the JE protein. PF-4 is present in platelet alpha granules and mast cell granules. To determine whether proinflammatory characteristics of PF-4 relate to induction of adhesion molecules for leukocytes, we examined the ability of PF-4 to elicit ICAM-1 in human endothelial cells *in vitro*. Immunohistochemical analysis of umbilical vein endothelial monolayers cultured for 6, 12, 24, 48 and 72 hours in medium alone or controls for PF-4 endotoxin content (0.5 EU/ml) revealed only trace reactivity for ICAM-1 in isolated cells. Replicate cultures exposed to PF-4 demonstrated strong ICAM-1 expression by small clusters of endothelial cells at 6 hours, diffuse reactivity by most endothelial cells by 24 hours, and dissipation to baseline levels thereafter. The pattern and kinetics of endothelial ICAM-1 expression was similar to but less intense than that induced by addition to cultures of recombinant interferon gamma (IFNγ) or IFNγ+interleukin 1 (IL-1). HLA-DR, but not HLA-DQ, was induced by IFNγ at 24 and 48 hours, and neither was elicited by exposure of cultures to PF-4. PF-4-induced ICAM-1 staining was qualitatively less intense when elicited by PF-4 carboxy-terminal synthetic peptide (41 amino acids), and was not observed after exposure of cultures to PF-4 amino-terminal synthetic peptide (29 amino acids), confirming functional activity to reside in the former, as determined by previous bioassays. These data suggest that recently described proinflammatory effects of human PF-4 may relate to induction of ICAM-1 in endothelial cells. In skin, aggregating platelets and perivascular mast cells are strategically positioned for rapid release of PF-4 in proximity to microvascular endothelium, suggesting a role for these cells in host defense against malignancy and infection.

IN VIVO ADMINISTRATION OF RECOMBINANT IL-2 INCREASE THE NUMBER OF EPIDERMAL LANGERHANS CELLS AND THY-1⁺ DENDRITIC EPIDERMAL CELLS. DIFFERENTIAL EFFECT ON YOUNG AND AGED MICE. Naotaka Shibagaki, Kunihiko Tamaki, and Shinji Shimada. Yamanashi Medical College, Yamanashi, Japan.

Recent studies show that immunological functions decrease with age. In the epidermis of mice, there are two types dendritic cells, one is Thy-1⁺DEC and the other is LC. In this study, we used epidermal sheets from mice stained with monoclonal antibodies to determine whether Thy-1⁺DEC's and LC's morphological structure and the number of cells differ between young (10W) and aged (24M) mice. We also investigated if the cell's number in young and aged mice can be increased by injecting rIL-2 in vivo, and if so, is there a difference. We found that 1, Thy-1⁺DEC and LC of aged mice and young mice were morphologically different and that aged mice had about 50% fewer cells than the younger mice. 2. when rIL-2 was injected into the abdominal cavity of young mice, the number of these cells increased 1.5-2 times in a time and dose dependent manner. All of Thy-1⁺DEC expressed CD3 and γ/δ TCR. 3. when rIL-2 was injected into left foot pad of the young mice, the cell number of the injected side increased more than the right side. 4. Although the number of these cells of aged mice increased, there was lower response in the earlier stages than that of the young mice. These studies demonstrated that aging changes the number and the morphological structure and the response of rIL-2 of the immunocompetent cells of skin.

CELL SURFACE EXPRESSION OF CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS BY MURINE EPIDERMAL LANGERHANS CELLS: A CONTROVERSY RESOLVED. Susan O. Sharrow, Alfred Singer, and Stephen I. Katz. Exper Imm and Derm Branches, National Cancer Institute, Beth, MD

Controversy exists regarding the expression of Class I major histocompatibility (MHC) antigens by murine epidermal Langerhans cells (LC). Some studies have reported very low levels of these molecules on freshly obtained LC (PNAS 81:3864, 1987, and JID 90:387, 1988) while others have reported significant levels (PNAS 86:7527, 1989). However, different Class I molecules were analyzed in each of these studies. The current study used quantitative immunofluorescence analysis to characterize cell surface expression by LC of several (H-2K, H-2D, H-2L and Qa) individual Class I molecules. We found that LC express H-2K at only 10-20% of the levels detected on splenic B cells. In contrast, LC express approximately twice the level of H-2D found on B cells. Keratinocytes were found to express both H-2K and H-2D at high levels (2-3 times those of B cells). The cell surface expression of Qa-2 by LC paralleled that of H-2K (and was low), while H-2L molecules were found to have a unique pattern of expression. Surface H-2L on LC was equivalent to that of B cells, whereas H-2L expression on keratinocytes was uniquely low (30-40% that of B cells). Thus, murine epidermal LC express low cell surface amounts of some, but not all, Class I MHC products. This differential expression of Class I MHC gene products probably explains the previously apparently contradictory studies and may reflect either specific regulation of individual Class I genes or post-translational events influenced by the developmental status of LC. Regardless of the mechanism, this differential expression may have consequences for Class I-restricted immune responses.

PURIFICATION OF HISTIDINE-RICH PROTEINS FROM RAT CORNIFIED CELLS. M. Shimozuma and K. Fukuyama, Department of Dermatology, University of California, San Francisco, CA.

Post-translational modifications of histidine-rich protein (HRP) and the resultant heterogeneity of their molecular weight (Mr) and isoelectric point (pI) have been demonstrated. We report purification and characterization of HRPs from 2-day-old rat cornified cells. Proteins of terminally differentiated keratinocytes were extracted in 0.1% acetic acid and dialyzed against pH 7.0 buffer. Soluble proteins were applied on a Sephacryl S-300 column. A protein fraction with apparent Mr>80k showed a typical amino acid composition of HRP and multiple bands of Mr 18-49k were identified by SDS-PAGE. The proteins were fractionated on a Mono S column equilibrated with pH 7.0 buffer by a NaCl gradient. HRP as determined by amino acid composition eluted at 0.125 M NaCl concentration was composed of several proteins with Mr 18-28k, but this HRP appeared as a single peak on a reverse phase Pro RPC column. Purification of proteins according to pI was then achieved by the use of a Mono P column and Polybuffer 96 on a FPLC system. By the chromatofocusing method, proteins with pI>9 and pI 7-6.5 were separated; the ratio of the two HRPs was about 4:1 respectively. By SDS-PAGE, 23k, 21k and 18k proteins were found in pI>9 HRP and 28k, 23k and 21k proteins in pI 7-6.5 HRP. These results suggest that the neutral buffer soluble HRPs exist in cornified cells and have Mr much lower than previously described. The members of the HRP family exibit isoelectric variance and some may lack the biological activities assigned to basic HRP.

EXPRESSION OF INTERLEUKIN 1 RECEPTORS IN HUMAN EPIDERMIS: INCREASED EXPRESSION IN PSORIATIC EPIDERMIS. L. Sherman, R. Ghiselli, and T.S. Kupper, Washington Univ. Sch. of Med., St. Louis, MO.

Cultured human keratinocytes (HK) contain mRNA homologous to the IL-1R expressed by T cells and fibroblasts, and can regulate their expression of IL-1R from fewer than 50 to greater than 50,000 sites per cell. Keratinocytes also produce functional IL-1 alpha, and interaction of IL-1 and IL-1R on HK leads to de novo production of cytokines which mediate chemotaxis and activation of leukocytes. It has been postulated that keratinocytes thus "activated" may orchestrate inflammatory events in skin. A method for visualizing specific IL-1R in fresh unfixed epidermis has been developed, using 125-I labelled IL-1 in the presence or absence of a 100-fold excess of unlabelled IL-1, followed by fixation, paraffin embedding and sectioning, and autoradiography. Normal human epidermis does not express significant numbers of IL-1R. Additional sites are not revealed after incubation at pH 3.0, which dissociates bound IL-1 from its receptor. After 24 hours in organ culture with various stimuli, markedly increased numbers of receptors were visualized in the basal and suprabasilar regions of epidermis. Freshly isolated lesional psoriatic epidermis, unlike either normal or non-lesional epidermis, shows significant expression of IL-1R on basal keratinocytes. The observation that IL-1 alpha is reduced in lesional skin (which we interpret as consumption of ligand), coupled with the expression of IL-1 inducible cytokines in lesional psoriatic epidermis (IL-6, IL-8), suggests that de novo expression of IL-1R in psoriatic epidermis is linked to pathophysiology in this disease.

THERE IS NO CIRCADIAN RHYTHM IN IMMUNE REACTIVITY TO CONTACT SENSITIZERS IN MICE. Gulnar M. Shivji and William R. Brown, Division of Dermatology, University of Toronto, Ontario, Canada.

To investigate possible circadian rhythms in immune reactivity to contact sensitizers we performed a series of 21 experiments in mice, using oxazolone and dinitrofluorobenzene (DNFB). We did 11 experiments with BALB/c mice, 5 with C57BL/6 mice, and 5 with SJL mice. Groups of 4 to 6 mice were used at 6 time points over 24 hours. In some experiments mice were resensitized with the other antigen after 6 weeks, and the groups were shifted by 12 hours. All mice were sensitized in the morning on the shaved back skin with either DNFB or oxazolone. Groups of mice were challenged on the ears with the appropriate contact sensitizer at each of 6 time points between the fifth and sixth days. Ear thickness was measured before challenge and 24, 36, and 48 hours later, to determine the peak ear swelling for each ear. The 21 experimental series gave inconsistent and irregular patterns of reactivity over 24 hours. The higher responding groups remained high responders despite being shifted by 12 hours. This negative finding is in contrast to our published findings of regular circadian rhythms in cell proliferation, and it differs from the one published study we found of a circadian rhythm in contact sensitivity to oxazolone in rats. Pownall and Knapp (1978) found a 9 fold drop in reactivity over 6 hours and an increase over 18 hours. Their study used 6 rats at each of 6 time periods whereas our combined studies gave us about 180 data points at each time point. We conclude that there is no circadian rhythm in immune reactivity to contact sensitizers in mice.

IDENTIFICATION AND CHARACTERIZATION OF NUCLEAR RETINOIC ACID-BINDING ACTIVITIES IN HUMAN CULTURED DIFFERENTIATING KERATINOCYTES. Georges Siegenthaler, *Marie-Pierre Gaub, Denis Salomon, *Arthur Zelent, Jean-Hilaire Saurat, *Pierre Chambon. *Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland and *Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg- Cédex, France.

It is suggested that the mechanism of action of retinoic acid (RA) are mediated by nuclear receptors (RARs), since RAR-α, -β and -γ -mRNA has been detected in human epidermal cells. Human foreskin keratinocytes (K) were cultivated in normal Ca until they form multi-layers, then differentiating cells were separated from non-differentiated by switching the culture in low Ca. The crude nuclear extracts from cultured differentiating and nondifferentiated K were analysed for specific (^{3}H)retinoic acid (RA)-binding sites by size exclusion HPLC and by PAGE techniques standardized with nuclear extracts of Cos cells transfected with the human RAR expression vectors RAR-α and RAR-β and RAR-γ, respectively and with purified human epidermal CRABP. We demonstrate that nuclear extract from differentiating K, when incubated with (^{3}H)RA shows two radioactive peaks at 50k Da and 15k Da which might correspond to RARs and CRABP. Using PAGE technique we show that RARs and CRABP migrate with the same mobility than the standards. The binding activities were about 0.1pmol/mg protein for RARs and 0.04pmol/mg for CRABP, with a RARs/CRABP ratio between about 2.5-1.4. These binding activities were not detected in nondifferentiated K, whereas RAR-α, -β, -γ -mRNA detected by Nothern-blot analysis were expressed in the two different cell populations. The expression of RARs and the presence of CRABP in the nucleus of differentiating K correlate with our previous findings of high level of cytoplasmic CRABP and the enzyme activity which transform retinol into RA in those cells. Thus, compared to the nondifferentiated, only differentiating possess all the metabolism pathway of retinoids suggesting that differentiating K might be the target cells for RA action.

DIFFERENTIAL TRANSCRIPTION, TRANSLATION AND ACTIVITY OF TYROSINASE DURING INDUCED HAIR GROWTH IN MICE. Andrzej Slominski, Ralf Paus*, Robert Costantino. Toolan Inst. for Med. Res., Bennington, VT and Dept. of Microbiology & Immunology, Albany Med. Col., Albany, NY; *Dept. of Dermatology, Yale University Sch. of Med., New Haven, CT.

In C57 Bl-6 mice, active melanogenesis is strictly coupled to the growth phase of the hair cycle (anagen). To elucidate the molecular mechanism of this phenomenon we have followed the sequence of tyrosinase expression and activity (key enzyme of melanogenesis) during the development of anagen follicles. Analyses were performed on protein extracts and RNA of whole mouse skins from telogen (day 0) and stripping induced anagen mice [day (d)1,2,3,5,8,12 after stripping]. On d0, tyrosine hydroxylase activity was very low and dopa oxidase (DO) activity was undetectable. On d1 & 2, both activities were undetectable, while starting from d3 they increased rapidly, reaching a plateau on d8 & 12. DO positive proteins had an apparent MW of 66 kD (d3-12), of 72 kD (d5-12), and of 130 kD (d8 & 12). Immunostains of proteins probed with polyclonal anti-tyrosinase antibodies (Arch Biochem Biophys 275:122, 1989) emphasized a protein of MW 66 kD, which was undetectable on d0, already present on d1 & 2, and abundant on d8 & 12. Northern blot analysis revealed low levels of tyrosinase mRNA on d1 & 3, high on d5 & 8, and none on d0. These data show a regulated, differential pattern of tyrosinase transcription, translation, isozyme repertoire and enzyme activity during the different stages of the developing murine anagen follicle, possibly as a result of complex bidirectional interactions between follicular melanocytes and their environment.

DIFFERENTIAL EFFECTS OF LOW-DOSE UVB-IRRADIATED LANGERHANS CELLS (LC) ON SUBSETS OF CD4$^+$ CELLS: INDUCTION OF UNRESPONSIVENESS OF TH_1 AND ACTIVATION OF TH_2. J.C.Simon, P.D.Cruz, Jr., P.R.Bergstresser and R.E.Tigelaar, Dept. of Dermatology, UT Southwestern, Dallas, TX.

UVB radiation distorts the capacity of LC to initiate delayed-type hypersensitivity (DTH) in vivo and to present soluble antigens(Ag) to immune T cells in vitro. UVB-induced suppression of DTH has also been shown to be mediated ultimately by CD8$^-$ (CD4$^+$) T cells. Two subsets of CD4+ T cells, TH_1 and TH_2 have been identified based on their cytokine production and function; in particular, TH_1 mediate DTH, whereas TH_2do not. To investigate whether UVB irradiated LC evoke differential effects on these subsets, we examined the capacity of unirradiated and irradiated (200 J/m^2) antigen presenting cells (APC) from BALB/c mice to present keyhole limpet hemocyanin (KLH) to Ag-specific, H-2d-restricted TH_1 and TH_2 cells. Four APC were utilized: epidermal cells (EC), FACS-purified Ia$^+$ EC (LC), FACS-purified Ia$^-$ keratinocytes (KC), and splenic adherent cells (SAC). Unirradiated EC, LC and SAC, but not KC, presented KLH to both TH_1 and TH_2. Irradiated EC and LC lost their ability to stimulate TH_1, but retained their capacity to stimulate TH_2. By contrast, irradiated SAC were unable to induce proliferation of either TH_1 or TH_2. To determine whether irradiated LC induced long lasting unresponsiveness in TH_1, we examined the effect of adding syngeneic unirradiated to TH_1 previously cultured with irradiated LC. Such TH_1 remained unresponsive to restimulation with normal APC. We conclude that low-dose UVB-irradiated LC may suppress DTH via two mechanisms: 1) induction of unresponsiveness in TH_1 cells and 2) unperturbed activation of TH_2 which may themselves mediate suppression.

SEPARATION OF NONCUTANEOUS EPITHELIA IN A FETUS AFFECTED WITH JUNCTIONAL EPIDERMOLYSIS BULLOSA. Lynne T. Smith, Steven Brumbaugh, Karen A. Holbrook. Department of Biological Structure, University of WA, Seattle, WA.

Junctional epidermolysis bullosa (JEB) is a severe blistering disorder with autosomal recessive inheritance. An affected fetus can be identified by fetal skin biopsy at 18-20 weeks of gestation. The present study was undertaken to identify noncutaneous epithelia that are involved during expression of this disease in utero. Skin and other organs from a 19-week estimated gestational age fetus affected with JEB and from age-matched controls were examined by light and transmission electron microscopy (TEM). Skin samples from different body regions including trunk, leg, arm, and finger, all showed some separation at the dermal-epidermal junction (DEJ). By TEM hemidesmosomes were absent or hypoplastic and separation was in the plane of the lamina lucida; anchoring fibrils appeared normal in structure and number. Interfollicular epidermis appeared to have separated easily, whereas some follicles remained anchored in the dermis. The stratified, nonkeratinizing epithelium of the tongue and inner lip, and the transitional epithelium of the bladder and urethra also showed separation. Pseudostratified ciliated columnar epithelium of the trachea was separated, but the glands appeared to remain attached to the connective tissue. Areas of the bronchial epithelium had separated, but within the lung parenchyma, the epithelium of smaller bronchioles and alveoli remained attached to supporting connective tissue. The gall bladder epithelium showed some separation. Epithelia which appeared to remain intact included the endothelium of large and small vessels in all organs and the linings of the stomach and the small and large intestines. Kidney, spleen, liver, and lymph nodes appeared normal in all aspects. In control samples, neither skin nor noncutaneous epithelia separated from the underlying connective tissue. The epithelial-mesenchymal separations observed in the fetus affected with JEB indicate that the expression of the abnormality occurs during fetal development and affects not only the integrity of skin, but also the epithelia of certain internal organs.

THE ADHESION MOLECULES CD11a, CD18, AND ICAM-1 ON HUMAN EPIDERMAL LANGERHANS CELLS (LC) SERVE A FUNCTIONAL ROLE IN THE ACTIVATION OF ALLOREACTIVE T CELLS. J.C.Simon, P.D.Cruz, Jr., R.D.Sontheimer, R.E.Tigelaar and P.R.Bergstresser, Dept. of Dermatology, UT Southwestern Medical Center, Dallas, TX.

Binding of antigen presenting cells (APC) to T-cells via adhesion molecules is thought to deliver accessory signals that are required for efficient T cell activation. To determine whether LC, considered the relevant APC within epidermis, express adhesion molecules on their surfaces, we employed a two-color immunofluorescence staining technique. Human epidermal cells (EC), Ficoll-enriched for LC (6-10%), were incubated with monoclonal antibodies (MAb) against the adhesion molecules CD11a (LFA-1α), CD18 (LFA-1β), or ICAM-1. LC were identified by MAb against HLA-DR, or CD1a. Staining was quantified by flow cytometry. After 18 h of culture only LC, among EC, constitutively expressed CD11a, CD18, and ICAM-1. To elucidate the function of these molecules, we tested the capacity of MAb against CD11a, CD18, or ICAM-1 to block the ability of LC to stimulate alloreactive T cells. EC were cocultured with allogeneic peripheral blood lymphocytes (PBL) for 5 days in the presence or absence of MAb; proliferation of PBL was measured by [3H]-thymidine uptake. MAb against CD11a, CD18, or ICAM-1 reduced by >70% the allostimulatory capacity of LC; combination of these MAb reduced proliferation by >90%, as did MAb against MHC class II. Inhibition was dose-dependent and specific, since control MAb had no effect. We conclude that interaction of adhesion molecules on LC with their ligands on T cells are required for optimal alloantigen-dependent T cell activation, perhaps by delivering accessory signals.

TYPE I AND VI COLLAGEN GENE EXPRESSION IN KELOID TISSUE. Stephan Sollberg, Juha Peltonen, Li Li Hsiao, Sirkku Jaakkola, Mon-Li Chu, and Jouni Uitto. Departments of Dermatology, Biochemistry and Molecular Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA.

Collagen gene expression was examined in 9 keloids utilizing molecular hybridizations with human collagen cDNAs. For spatial localization of gene expression, in situ hybridizations were performed on frozen sections with ^{32}P-labeled cDNAs. Autoradiography of [^{32}P]cDNA/mRNA hybrids revealed a large number of cells actively expressing α1(I) and α2(VI) collagen genes. These cells were largely present at the periphery of the expanding lesion, suggesting active collagen synthesis at these sites. Also, collagen mRNAs were detected in high abundance at the proximity of blood vessels within the keloids. For quantitative analysis, total RNA was isolated from keloid tissue, and the relative quantities of α1(I), α2(I), α1(VI), α2(VI) and α3(VI) collagen mRNAs were determined by slot-blot hybridizations. The results indicated that the ratio of type I/VI collagen mRNAs was ~5:1, and the ratio of α1:α2:α3 chain mRNAs of type VI collagen was 1:1.8:1.6. The latter value is at variance with determinations in cultured normal human skin fibroblasts which show a ratio of approximately 1:1:1. Also, indirect immunocytochemistry with a polyclonal anti-type VI collagen antibody revealed the presence of corresponding epitopes within keloids. The results indicate active expression of type I and VI collagen genes primarily at the periphery of the keloid lesions and suggest that these collagens are a significant component of keloids.

L-LEUCYL-L-LEUCINE-O-METHYL ESTER (LEU-LEU-OME) PRETREATMENT INHIBITS THE MIXED EPIDERMAL CELL-LYMPHOCYTE REACTION (MECLR).

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LEU-LEU-OME is converted to a toxic metabolite by the lysosomal thiol protease, dipeptidyl peptidase I (cathepsin C), which is highly enriched in susceptible cells such as macrophages, natural killer cells and cytotoxic T cells. Since the toxic metabolite results in rapid cellular death, LEU-LEU-OME has been used to deplete susceptible cell types from mixed cell populations. While examining the alloantigen presenting cell type(s) present in newborn human foreskin dermal cell suspensions, we observed in control experiments that LEU-LEU-OME pretreatment markedly diminished the ability of newborn foreskin epidermal cell (EC) suspensions to function as stimulators in a primary one-way, allogeneic MECLR. Responder peripheral blood mononuclear cell blastogenesis, as determined by tritiated thymidine incorporation after 6 days in culture, was decreased by 94%, 100%, 100%, 83%, 33%, 70% in 6 consecutive experiments when the stimulator EC suspensions had been pretreated with 250 micromolar LEU-LEU-OME for 15 minutes followed by a 2 hour incubation period. Similar results were obtained when highly purified T cells were used as responder cells. EC viability was not significantly diminished by LEU-LEU-OME treatment. These studies suggest that epidermal Langerhans cells (ELC), the relevant alloantigen-presenting EC type in a MECLR reaction, is susceptible to the toxic effects of LEU-LEU-OME. Flow cytometric studies are in progress to assess the effect of LEU-LEU-OME on ELC viability.

LIPID MODULATION OF KERATINOCYTES AFFECTS PROTEIN KINASE C (PK-C) TRANSLOCATION, CELL ATTACHMENT AND PROLIFERATION.

Ruth Steingart[*], Amos Simon[*], Beno Michel[**] and Yoram Milner[*]. The Dept. of Biol. Chemistry, the Hebrew University, Jerusalem, Israel[1] and CPI Laboratories Beachwood, OHIO, USA[2].

We have shown that protein kinase C is involved in initial phases of keratinocytes proliferation. PK-C translocation is the initial and crucial event in these phases. We have tested the effect of lipid modulation on these processes. Cultured human keratinocytes and cell lines (immortalized HACAT and carcinoma SCL-1) were grown for 24-28 hrs. with either 3% poly-vinyl-pyrrolidone (PVP) or 3% PVP + 100 µgr/ml cholesterol hemisuccinate (CHS) in order to deplete cholesterol (CH) or enrich cells with CHS respectively. Also, the cultures were enriched with 50µM of either palmitic, stearic, linoleic or linolenic acids. The depletion or enrichment were monitored by TLC separation of cells' lipids and densitometry and by direct determination of CH/CHS/phospholipid ratio. Fatty acids (FA) incorporation was evaluated by ^{14}C labeled FA in triglycerides and phospholipds or saponification and GS of FA methylesters. Microviscosity of cell membrane was determined by DPH photopolarization. We found that in "fluidized" cells (depletion of CH or unsaturated FA enrichment) PK-C is 60-90% translocated to the membrane and cells are adhering and proliferating faster than controls. In "Rigidified" cells (CHS, saturated FA enrichment), PK-C translocation is inhibited and there is an inhibition of cell attachment and proliferation.

Sunscreen Protection Against UVA And UVA+UVB Effects On Langerhans Cells And Keratinocytes in Human Subjects

J.W. Stanfield, V.J. Stevens, P.M. Pasciak, L. Daniels and Lincoln Krochmal, Bristol-Myers Squibb Pharmaceutical Research & Development, Buffalo, NY

Volunteers with skin types I or II were irradiated with either UVA (5 subjects) or UVA+UVB (5 subjects) to determine their MEDs on the lower back. A high SPF sunscreen (SPF 29) or its vehicle were then applied at 2 microliters/cm² and 3 MEDs were administered to sunscreen-protected, vehicle-protected and unprotected sites. After 24 hours superficial shave biopsies were obtained from each treated site and a control. Biopsy specimens were divided and 1/3 was incubated with tritiated thymidine, fixed, sectioned and developed by autoradiography; 1/3 was treated with fluorescein-conjugated OKT-6 antibody and 1/3 was stained with hematoxylin-eosin. Blinded slides were examined at 400x to count cells which incorporated thymidine, $T6^+$ (Langerhans) cells or sunburn cells.

Both UVA and UVA+UVB produced diminution of $T6^+$ cells, increased thymidine uptake and observable sunburn cells. For both UVA and UVA+UVB, the sunscreen protected against these effects. A larger sample size would be required to permit statistical significance of results.

THE SIGNAL FOR INDUCING HAIR GROWTH IS LONG-LIVED: THE EFFECT OF GLUCOCORTICOIDS ON HAIR GROWTH INDUCTION IN THE MOUSE.

KS Stenn, R Paus & T Dutton, Department of Dermatology, Yale University, School of Medicine, New Haven, CT.

The mechanisms initiating and regulating the cyclic nature of hair growth remain unknown. Using hair pluck as initiating stimulus and the C57 B16 mouse we have characterized the hair cycle using an ex vivo kinetic assay of follicle growth. With this assay maximal growth occurs at about three days post pluck decreasing in late anagen and catagen. Although plucking did not induce a significant inflammatory cell infiltrate, the development of anagen was blocked by topical glucocorticoids (GCs). The GC-induced block is complete for at least 6 days; namely, the follicles remain unequivocally in telogen (resting stage) grossly, histologically, and kinetically. Upon discontinuing the GCs after 6-14 days, however, the follicles progress into full anagen. With continuous GC application, there is a gradual escape from the block after about day 8; moreover, during the first 4 days the follicles are most sensitive and thereafter rather insensitive to steroid suppression.

These studies describe one characteristic of the traumatic stimulus of hair growth induction--it is not readily turned off. It appears once stimulated the switch is irreversibly turned on and the cycle is activated even if anagen development is suppressed. Since the GC-suppression of follicle growth occurs distal to the initiating event, this system may help elucidate the molecular events important to anagen induction.

UV-B INHIBITS GAP JUNCTION MEDIATED DYE TRANSFER IN NORMAL HUMAN EPIDERMAL KERATINOCYTES.

M. Steinberg*, S. Jadlos and K. Marenus, *Department of Chemistry, City College/New York, NY and Estee Lauder R&D, Melville, NY.

We have found that UV-B has a significant impact on gap junction mediated dye transfer. Monolayer cultures of normal human epidermal keratinocytes were grown to confluence and subjected to doses of UV-B irradiation (375, 750 and 1500 mJ/sq cm) from an FS-20 source. Immediately after irradiation; Lucifer Yellow, a low molecular weight fluorescent dye was introduced into the monolayer through cells adjacent to a scalpel wound edge. After 3 minutes incubation with the dye, the cultures were fixed and photographed under UV illumination. Results indicated a significant reduction in the distance and amount of dye transfer in response to the UV dose. In unirradiated cultures, the dye front could be detected more than 20 cell diameters distal from the wound edge. In irradiated cultures, a dose response relationship between UV and total dye transfer was observed.

UV-B Dose (mJ/sq cm)	% Reduction of Dye Transfer
375	47.3
750	56.5
1500	85.9

These initial experiments indicate a relationship between UV exposure and reduction of gap junction mediated material transfer.

TGF-BETA IS TOPICALLY EFFECTIVE AT REDUCING EPIDERMAL PROLIFERATION.

Victor Stevens, Lisa Daniels and Hemanshu Shah, Depts of Cell Biology and Preformulation, Bristol-Myers Squibb Dermatology, Buffalo, New York.

TGF-beta exerts an inhibitory effect on epidermal proliferation in culture. To assess its activity in vivo for therapeutic potential,in dermatology, the tape-stripped hairless mouse model was used. Skh-1 hairless mice were stripped of the stratum corneum along the dorsal flank with five applications of cellophane tape. TGF-beta or vehicle was applied three times over 21 hours at concentrations from 0.1 to 10 mcg/ml. Vehicle control sites were found on the controlateral side. At 22 hours the mice were injected with 25 mcCi of tritiated thymidine and sacrificed 2 hours later. 15mm diameter circles were punched from the treated areas for analysis. The epidermal cells were harvested after trypsin treatment to remove the S. corneum.

A dose response effect of TGF-beta on epidermal cell incorporation of thymidine was seen from 0.1 to 10 ug/ml TGF-beta. The maximal decrease was 50% of the untreated stripped site at 10 ug/ml. In vitro penetration studies showed a limited penetration of I-125-labeled TGF-beta across mouse epidermis. This was enhanced over 10 fold by tape stripping the skin. TGF-beta was more effective than anthralin, methotrexate or hydrocortisone in its anti-proliferative effect.

VERY LONG-CHAIN FATTY ACIDS IN CHILDREN'S SEBUM. Mary Ellen Stewart and Donald T. Downing, Department of Dermatology, University of Iowa College of Medicine, Iowa City, Iowa

Cholesterol esters (CE) with very long-chain (>18 C) fatty acids have been reported to occur in substantial amounts in fetal sebum (vernix caseosa), but are only a minor component of adult human sebum. Because, in some respects, the composition of fetal sebum resembles that of prepuberal children, we examined children's sebum for the presence of CE with very long-chain fatty acids.

Sebum was extracted from the hair of 12 children, aged 6 and 7, and part of the sample was fractionated by preparative thin-layer chromatography and column chromatography on $Mg(OH)_2$ to obtain pure CE. The CE were hydrolyzed, the freed fatty acids were converted to fatty acid methyl esters (FAME), and the FAME were analyzed by capillary gas chromatography to determine the percentage with more than 18 C. FAME from 5 of the subjects were then separated into saturated and monounsaturated fractions by the bromomercuriacetate method and analyzed again by gas chromatography to distinguish branched from straight chains. Aliquots of unfractionated sebum were analyzed by quantitative thin-layer chromatography to determine the ratio of wax esters/[cholesterol+cholesterol esters] (WE/[CH+CE]), a measure of sebaceous cell lipogenesis.

Fatty acids with more than 18 C ranged from 15 to 72% of CE FAME, with the highest values being found in the children with the lowest WE/[CH+CE]. The saturated FAME were mostly iso or anteiso branched, while the monounsaturated were mostly straight-chain extension products of 16:1Δ9 or 18:1Δ9. The composition of children's CE FAME may reflect a low level of endogenous lipid synthesis.

NEUTROPHIL-DERIVED OXIDANTS INACTIVATE TISSUE INHIBITOR OF METALLOPROTEINASES. George P. Stricklin and John R. Hoidal, Division of Dermatology, Vanderbilt University School of Medicine, Nashville, TN and Department of Medicine, University of Utah, Salt Lake City, Utah.

The almost ubiquitous presence of tissue inhibitor of metalloproteinases (TIMP) forms a barrier which must be overcome for extracellular matrix degradation to occur. We examined the ability of neutrophil-derived oxidants to degrade TIMP. Preliminary studies of chemical and enzyme produced oxidants demonstrated that changes in the immunoreactivity of TIMP were positively correlated with loss of TIMP function. Using immunologically based assays, we determined that normal neutrophils stimulated to release oxidants were very effective inactivators of TIMP. The inclusion of oxidant scavengers including methionine and catalase prevented TIMP degradation. Moreover, oxidant deficient neutrophils obtained from a patient with chronic granulomatous disease proved incapable of degrading TIMP. These data suggest that oxidant-mediated degradation of TIMP forms a prominent mechanism for the removal of this important barrier to matrix destruction.

IDENTIFICATION OF EGF RECEPTORS IN PSORIATIC SCALES USING A HIGHLY SENSITIVE ELISA ASSAY. C M Stoscheck, L B Nanney, and L E King, Jr. Veterans Administration and Vanderbilt University, Nashville, TN.

In a previous study using binding techniques and immunolocalization, we showed that the number of EGF receptors was elevated in psoriatic lesions (Nanney et al. 1986, JID 86:260). Both procedures were limiting in that binding studies can be effected by receptor accesability and affinity and immunolocalization studies are only semi-quantitative. Now we report the establishment of an ELISA assay which can measure as few as 1 fmol EGF receptors. When the ELISA assay was used to compare the number of EGF receptors in psoriatic scales and callus from normal patients, the number of EGF receptors in psoriatic scales was higher than normal comparable epidermis. These observations support our earlier reports of persistence of the EGF receptors into the outer parakeratotic layers of active psoriatic lesions. In both types of outermost epidermis, the number of EGF receptors was lower than the amount measured in thin skin (which contains the EGF receptor rich stratum basalis, Nanney et al. 1984, JID 83:385). This indicates that some, albeit a reduced amount of down regualation of EGF receptors has occured in the outermost layers of the psoriatic lesion. In conclusion, this highly sensitive ELISA assay allows the analysis of changes in EGF receptor numbers in the outermost layer of skin. Thus relatively non-invasive epidermal sampling techniques such as brushing, peeling or tape stripping can be used to measue EGF receptor levels. EGF receptor number may be a good method to monitor disease progression or therapies involving skin.

CHARACTERIZATION OF A 40K LOW-SULFUR HAIR KERATIN. Steven Sundby, *Scott Panter, Carol Niemi, and Maria Hordinsky, Department of Dermatology, University of Minnesota Medical School, Minneapolis, Minnesota, and the *Department of Neurology, Veterans Administration Hospital and University of California, San Francisco, California.

The "low-sulfur keratins" (40-70K) represent one of the three major classes of hair proteins. There are at least 5 different keratins commonly present in this region, two acidic or type I keratins of 44K and 46K, and three basic or type II keratins of 56K, 59K, and 60K. An additional protein of approximately 40K is occasionally present in human hair and when present, the 46K keratin appears to be reduced in amount such that the 46K and 40K proteins are about equal in quantity. In this study, we examined the heterogeneity in the low-sulfur keratins of human hair. We studied hair samples from 659 individuals (351F, 308M) by solubilizing the samples using 8 M urea, 20 mM dithioerythritol, pH 8.0, and alkylating with 0.14 M iodoacetamide prior to analysis using polyacrylamide gel electrophoresis and immunoblotting with the "AE" (Antibodies to Epithelial Antigens) monoclonal antibody series. Five AE antibodies were used: AE1, AE3, and AE2 directed against acidic soft keratins or basic keratins or against the 56.5K/65-67K skin-type differentiation keratins respectively, AE13, directed against the acidic 44K/46K keratin doublet, and AE14, directed against the 10-25K "high-sulfur" proteins. We found the 40K protein to be present in 34 (19F, 15M) of 659 or 5% of the individuals studied. The 40K protein was not recognized by AE1, AE2, AE3, or AE14. However, AE13 recognized the 44K/46K acidic keratin doublet, as expected, as well as the 40K protein, placing it in the family of the acidic "hard" keratins found in human hair and nails. The distribution of the 40K hair keratin by sex and age was also examined. We found no statistical correlation with sex but did find that the 40K keratin is not present at the 5% level for all age groups ($p<.005$) and is predominantly seen in a younger population.

IMMUNOGENETIC SUSCEPTIBILITY TO UVB AS A RISK FACTOR FOR SKIN CANCER IN MAN. J. Wayne Streilein, T. Yoshikawa, V. Rae, and J. Richard Taylor, Departments of Microbiology/Immunology, and Dermatology, University of Miami School of Medicine, Miami, Florida

Experiments have been conducted in caucasian human subjects (color types II, III) to explore the putative etiologic relationship between the deleterious effects of UVB irradiation on cutaneous immune responses, and the risk of developing skin cancer. A universally sensitizing dose of DNCB (2000 ug) was used to induce contact hypersensitivity (CH) in normal healthy volunteers (34) and in patients (10) with biopsy proven history of skin cancer (basal and squamous cell). DNCB was applied to untanned buttock skin immediately following an acute, low dose UVB irradiation protocol (144 mJ/cm^2 x 4 days) that virtually eliminates all normal Langerhans cells from the treated site. Among UVB-treated healthy volunteers, 60% developed vigorous CH while the remainder displayed neither primary allergic reactions, nor CH at forearm challenge sites. By contrast, 90% of UVB-treated skin cancer patients failed to develop CH. UVB-susceptible individuals (who failed to develop CH after UVB) were then subjected to a second immunizing regimen with DNCB (without UVB). All healthy volunteers developed CH, indicating that the initial failure to respond to DNCB via UVB-treated skin was an immunologically "null" event. Among UVB-susceptible skin cancer patients, 50% did not develop CH, even after the second sensitizing dose of the hapten, indicating that the first encounter with DNCB had induced immunologic tolerance. We conclude that human beings display a polymorphism for the effects of UVB on the induction of CH. Since the incidence of the UVB-susceptibility trait is significantly higher among skin cancer patients, and includes the tendency to become tolerant, we propose that UVB-susceptibility may be a risk factor for the development of skin cancer.

CELL VOLUME DECREASE DURING CHOLINERGIC STIMULATION INVOLVES THE Ca-DEPENDENT EFFLUX OF CELLULAR KCl. Y. Suzuki, M. Ohtsuyama, G. Samman, F. Sato, and K. Sato. Marshall Dermatology Research Lab., Univ. of Iowa College of Medicine, Iowa City, IA.

Eccrine sweat secretion may involve a number of cellular and membrane processes but their details are far from clear. Our recent X-ray microanalysis studies have suggested that cell shrinking may occur during pharmacological stimulation but the direct proof has been lacking. Using the video system on a microscope, we have now confirmed that collagenase digested dissociated monkey eccrine clear (but not dark) cells indeed shrank as much as 35% during MCh stimulation. MCh-induced cell shrinking (MICS) peaks within 15 seconds after stimulation and is followed by partial volume recovery in the continuous presence of the drug. In order to determine the ionic basis of MICS, the effect of varying [K] and [Cl] was studied. At 120 mM KCl (which reverse the direction of net chemical potential for KCl), MICS was nearly completely abolished but was restored when Cl was replaced by impermeant anion methylsulfate, suggesting that the diffision of K and Cl through the respective channels is critical for MICS. In deed, MICS was completely (reversibly) blocked by 1 mM quinidine (a K-channel blocker) and partially inhibited by 1 mM DPC (a Cl channel blocker). MICS was also completely inhibited by removal of Ca from the perfusate but was not blocked by the Na-pump inhibitor, ouabain, or the inhibitor of Na/K/2Cl cotransporter, bumetanide (both at 0.1mM). Other agents that are known to elevate the intracellular [Ca] such as ATP and ionomycin also induced cell shrinking although to a lessor degree whereas the cAMP elevating agent, isoproterenol, failed to induce cell shrinking. We conclude that cell shrinking is a dynamic initial cellular response of eccrine clear cells to cholinergic stimulation and is mediated by activation Ca-sensitive K and Cl channels.

THE ULTRASTRUCTURE OF LIPID LAMELLAE IN AVIAN EPIDERMIS Donald G Swartzendruber, David P Boysen, Philip W Wertz, and Donald T Downing, Dermatology Department, University of Iowa, Iowa City, IA

Avian epidermis is known to produce very thin corneocytes with little or no intercellular lipid lamellae. Lamellar bodies are abundant in the cells of the granular layer and in the transitional cells, but are reported to be retained within the cells as they cornify. In the present study we used electron microscopy to examine sections of chicken epidermis postfixed with ruthenium tetroxide, which in mammalian stratum corneum reveals intercellular lamellae that are not revealed by the conventional osmium tetroxide reagent. We found no evidence of intercellular lipid lamellae, or their formation from extruded lamellar granules as occurs in mammalian skin. Extraction of chicken stratum corneum with chloroform:methanol (C:M) removed 42.5% by weight of lipids, predominantly diol diesters, triglycerides, cholesterol, hydroxyacid derivatives, and cholesteryl glucoside. Electron micrographs of the extracted SC showed pairs of lipid envelopes between cells. After fine grinding, C:M extracted a further 6.4% of lipid that contained mainly cholesteryl esters and triglycerides. Subsequent mild saponification and extraction removed bound lipids, amounting to 6% of the original tissue, containing only lipids more polar than cholesterol. We conclude that the stratum corneum barrier in chicken epidermis resides predominantly in lipid lamellae which form a central, lamellar structure within each corneocyte, and in a lipid envelope attached outside the protein envelope of each corneocyte.

ACTION SITE OF EXFOLIATIVE TOXIN ON KERATINOCYTE. Y Takagi, S Futamura, Y Asada. Dept. of Dermatology, Kansai Medical Univ., Osaka, Japan

The Staphylococcal scalded skin syndrome (SSSS) is caused by exfoliative toxin (ET). The purpose of the present study is to investigate the action site of ET on keratinocyte by immuno-EM and immunoblotting.

ET was applied to the normal human epidermal organ culture, which was incubated for 1.5, 2.5, and 3.5 hours at 37°C, and specimens were studied by electron microscopy(EM), immuno-EM and immunofluorescence(IF). ET binding proteins were also analyzed by immunoblotting. By ET, acantholysis was observed at the level of granular cell layer (GC). Intercellular fluorescence (ICS-F) was first observed in the basal cell layer and then in spinous cell layer and GC in the course of time by IF. Acantholysis occurred when ICS-F was seen at GC. By immuno-EM, gold particles were mainly seen in the outer leaflet of desmosomes, but sometimes in the intercellular contact area, attachment plaque and tono-filaments inserting into desmosomes. By immunoblotting, ET bound to 165KD in murine epidermal extract.

These results indicate that ET attaches to 165KD which may be desmoglein I. Further this study supposes the idea that acantholysis in SSSS, at least in part, from a ET-induced disruption of desmosomes.

MICROVASCULAR ENDOTHELIAL CELL VITRONECTIN RECEPTOR MEDIATES BOTH CELL-MATRIX AND CELL-CELL ADHERENCE. R Swerlick, E Garcia-Gonzalez, E Brown, T Lawley, Emory Univ, Atlanta, GA, Wash Univ, St Louis, MO.

We have recently shown by flow cytometry and immunoprecipitation that human dermal microvascular endothelial cells (HDMEC) express a cell surface protein complex identical to the vitronectin receptor (VnR). However, the function of this receptor is unknown. In order to determine whether this complex plays a role in cell-matrix and cell-cell interactions, we used both a HDMEC-matrix adherence assay and a T cell-HDMEC binding assay. Non-tissue culture plastic was treated with 10-50 ug/ml of either vitronectin (Vn), fibronectin (Fn), type 1 collagen (C1), or fibrinogen (Fb). HDMEC radiolabelled with ^{51}Cr were allowed to adhere in the presence or absence of monoclonal antibodies (Mab) to the vitronectin receptor. Mab 3F12, which recognizes the alpha chain of the VnR blocked adherence of HDMEC to Vn, but not to Fn, Fb, or C1. Mab 7G2, which recognizes the VnR beta chain, did not block adherence to any substrate. The adherence of HDMEC to Vn was blocked by RGD peptide at concentrations as low as 100 ug/ml, but required at least 500 ug/ml to block adherence to Fb or Fn. T cell binding to HDMEC monolayers was measured using radiolabelled T cells. Mab 3F12 blocked binding of T cells to HDMEC by 31±5% at a concentration of 20 ug/ml, blockade equivalent to that seen using antibodies to ICAM-1 (30±10%). RGD peptide (500ug/ml), active in inhibiting HDMEC binding to matrix, did not inhibit T cell-HDMEC binding. Since 3F12 does not bind to T cells, this data suggests that the HDMEC VnR may play an important role in both HDMEC-T cell binding and HDMEC-matrix interactions. Furthermore VnR mediated cell-matrix interactions are dependent on recognition of RGD sequences but VnR mediated T cell-HDMEC binding is RGD independent.

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GMCSF) IN BEHCET'S SYNDROME. Hideto Takahama, Shuichi Furusawa, Rie Watanabe, Chiyuki Inoue, Masako Mizoguchi, Department of Dermatology, Teikyo University School of Medicine, Tokyo, Japan.

In Behcet's syndrome (BS), increase of peripheral polymorphonuclear neutrophils (PMN), activated function of PMN, and lesions with infiltration of PMN are often found. Thus, PMN may play an important role in pathogenesis of BS. Recently, it was reported that GMCSF, a family of hematopoietic growth factors, enhanced PMN activity. To find out the role of GMCSF in BS, we first performed the polarization assay of peripheral PMN using N-formyl-L-methionyl-phenylalanine (FLMP) and GMCSF as chemotactants and calculated the percentage of polarized PMN. The mean of the percentage to FLMP for 12 patients was higher than that for 11 normal controls, which certified the patients had activated PMN. However, the mean of the percentage to GMCSF for the patients was lower than that for the controls, suggesting that PMN of the patients had been already activated in vivo by GMCSF and that the PMN did not react to GMCSF in vitro. So, we examined serum levels of GMCSF by RIA, however, the levels of both patients and controls were lower than the detectable level for RIA. Then, to find out the condition of GMCSF production, we examined mRNA of peripheral mononuclear cells by the dot blot analysis, which showed higher levels of mRNA in the patients than in the controls. It is concluded that GMCSF was actively produced in BS patients and activated PMN in vivo. Finding factors which enhance GMCSF production is important for studying the pathogenesis of BS.

IL-1α, IL-6 AND TUMOR NECROSIS FACTOR-α ARE PARACRINE INHIBITORS OF HUMAN MELANOCYTE PROLIFERATION AND MELANOGENESIS. Viki Swope, Zalfa Abdel-Malek, James Nordlund, Dept of Dermatology, Univ of Cinti College of Med, Cinti, OH.

The epidermal cytokines interleukin (IL)-1α, IL-6 and tumor necrosis factor-α (TNF-α) are known to produce many similar biologic effects. The possible paracrine effects of these cytokines on melanocyte proliferation and melanization have not been investigated before. Thus, in this study, we examined the effects of human recombinant IL-1α, IL-6 and TNF-α on normal human melanocytes (NHM). IL-1α, IL-6 and TNF-α elicited a dose dependent decrease in the activity of the enzyme tyrosinase after 48 hours of treatment. IL-1α at 0.05, 0.5 and 5 U/ml inhibited tyrosinase activity by 22%, 44% and 63%, respectively. Similarly, IL-6 at 10, 100 and 300 U/ml decreased tyrosinase activity by 23%, 33% and 42%, respectively. TNF-α at 10^{-9} M and 10^{-8} M also resulted in a 46% and 67% inhibition of tyrosinase activity, respectively. IL-1α, IL-6 and TNF-α produced a dose dependent decrease in the rate of ^{3}H-thymidine incorporation by NHM. All three cytokines increased the doubling time of NHM. The effects of IL-1α and TNF-α were cytostatic not cytotoxic since NHM remained viable after 7 days of cytokine treatment and resumed proliferation after the cessation of treatment. These effects of IL-1α, IL-6 and TNF-α do not seem to be mediated by the stimulation of eicosanoid production, since inhibition of arachidonic acid metabolism into cyclooxygenase or lipoxygenase products did not reverse the cytokine inhibitory effects. In conclusion, IL-1α, IL-6 and TNF-α, cytokines synthesized by keratinocytes and Langerhans cells, act as downregulators of melanin synthesis and proliferation of epidermal melanocytes. The exact mechanism(s) of action of these cytokines is currently under investigation.

THE HEMATOXYLIN STAINABLE KERATOHYALIN GRANULE PROTEIN IS A CYSTEINE PROTEINASE INHIBITOR. Masae Takahashi and Tadashi Tezuka, Department of Dermatology, Kinki Univ. School of Medicine, Osaka, Japan

We previously purified a buffer soluble, acidic hematoxylin stainable protein (HSP), which located on the cell membrane region of the stratum corneum, from newborn rat epidermis. In this study, we investigated both the origin of HSP using a modified fixation and embedded methods and the biological properties of HSP as a cysteine proteinase inhibitor. HSP was purified from 3-day-old rat epidermis by preparative isoelectric focusing, Superose 12 and Mono Q columns, and then a polyclonal antibody (PoAb) against HSP was produced. A newborn rat skin was fixed in 0.025% glutaraldehyde and 2% paraform-aldehyde. After a rinse, it was embedded in Lowicryl K4M. The microtome section was performed by an indirect immunofluorescent study using the PoAb. As the amino acid composition of HSP was similar to the one of cystatin α, the inhibitory activity of HSP was assayed using papain, ficin, trypsin, chymotrypsin, pepsin, and thermolysin. The inhibition constant (ki) was determined using BAPNA. HSP originated in the keratohyalin granules (KHG). HSP inhibited the activity of papain and ficin, but not that of non-cysteine proteinases. HSP was stable at various temperatures and pHs. The ki value against papain was 270 nM. The immunoblotting study using the PoAb showed that while it cross-reacted with cystatin α, the pI of HSP was different from the one of cystatin α. Therefore, HSP, one of the KHG component proteins, was a cysteine proteinase inhibitor, which could be related to cystatin α, and which might inhibit the bacterial cysteine proteinases.

RESPONSE OF SCLERODERMA FIBROBLASTS TO VARIOUS GROWTH FACTORS. Kazuhiko Takehara, Yoshinao Soma, Atsuyuki Igarashi, Kanako Kikuchi, and Yasumasa Ishibashi, Department of Dermatology, Faculty of Medicine, Univ. of Tokyo, Tokyo, Japan.

Abnormal growth regulation in lesional skin fibroblasts may be part of the pathogenesis of scleroderma. Abnormal response of cultured fibroblasts derived from sclerotic lesions to growth factors has been reported.

We investigated response of scleroderma skin fibroblasts (7 strains) and normal skin fibroblasts (6 strains) to various growth factors such as PDGF, TGF-β, EGF, and basic FGF. Experiments were conducted at confluent and growth stages. PDGF, EGF, and basic FGF stimulate fibroblast growth during the confluent and growth stages; however, response of scleroderma fibroblasts was significantly lower than that of the normal fibroblasts. TGF-β slightly stimulated confluent fibroblast growth and inhibited subconfluent fibroblast growth, but scleroderma fibroblast response exceeded that of normal fibroblasts.

The decreased response to growth-stimulating factors observed in scleroderma fibroblasts suggests in vitro-cultured fibroblasts derived from scleroderma lesions were already senescent because they had been activated by growth-stimulating factors and divided repeatedly in vivo. Thus, abnormal growth regulation of skin fibroblasts may be part of the pathogenesis of scleroderma.

cDNA CLONING OF BULLOUS PEMPHIGOID (BP) ANTIGEN REVEALS STRUCTURAL AND SEQUENCE HOMOLOGY WITH DESMOPLAKIN (DP) I. Toshihiro Tanaka, David A. D. Parry, Neil J. Korman, Vera Klaus-Kovtun, Peter M. Steinert, and John R. Stanley, Dermatology Branch, NIH, Bethesda, MD and Massey Univ., Palmerston North, New Zealand.

Using a primer from near the 5' end of a previously isolated 2 kb partial cDNA for the 230 kD BP antigen, we constructed a cDNA library in λgt11 from keratinocyte mRNA. This library, and an oligo-dT primed λgt11 library, were screened by hybridization with a probe from the furthest 5' end of the initial BP cDNA to isolate BP cDNAs which cover 4.8 kb. Nucleotide sequencing revealed a 4179 bp open reading frame, encoding a peptide of 160 kD ending at the carboxy (C)-terminus. Because protein A-gold immunoelectron microscopy revealed that antibodies raised to peptides encoded by this cDNA bind to the dense plaque of the hemidesmosome, we compared the peptide sequence of BP antigen to that of DPI (Green KJ et al, J Biol Chem, in press), which is present in the dense plaque of the desmosome, and found striking similarities. Towards the amino (N)-terminus, BP antigen, like DPI, has the amino acid heptad repeat typical of α-fibrous proteins that form a coiled-coil dimer. Comparison of the C-terminus of BP antigen with that of DPI revealed a 39% identity and 61% homology over 521 amino acids, with both molecules having almost identical 38 residue internal repeating motifs. Finally, as in DPI, charge periodicity of this area of BP antigen is similar to that of keratin filaments, suggesting a potential means of interaction. These findings suggest that there is a family of adhesion junction plaque proteins, which includes BP antigen and DPI.

BRADYKININ, HISTAMINE AND PLATELET ACTIVATING FACTOR STIMULATE RELEASE OF ARACHIDONIC ACID AND PROSTAGLANDIN E_2 FROM ADULT HUMAN KERATINOCYTES. Harvinder S. Talwar, Gary J. Fisher, John J. Voorhees, Department of Dermtology, University of Michigan Medical School, Ann Arbor, MI.

Bradykinin (BK), histamine (HIS), and platelet activating factor (PAF) induce an inflammatory response when injected into the skin. Previous studies have demonstrated that specific cell surface receptors for these inflammatory mediators are present on cultured adult human keratinocytes and are coupled to phospholipase C-catalyzed phosphoinositide turnover, which generates inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 raises intracellular Ca^{2+} and DAG activates protein kinase C. In other cell types, it has been shown that activation of phosphoinositide turnover is associated with mobilization of arachidonic acid from membrane phospholipds and increased eicosanoid synthesis. The present studies were conducted to investigate whether BK, HIS, and PAF are capable of inducing the release of arachidonic acid and its metabolite prostaglandin E_2 (PGE_2), in cultured adult human keratinocytes. Arachidonic acid mobilization was assessed by release of label, into the media, from cells prelabeled with [^{3}H]arachidonic acid and PGE_2 was measured by radioimmunoassay. Addition of BK, HIS, or PAF to keratinocytes elevated arachidonic acid and PGE_2 release 3-fold within 30 minutes (N=5). Half-maximal and maximal release of PGE_2 occured at 10nM and 100nM BK, respectively. Maximal increases in PGE_2 release by HIS and PAF were observed at 100μM and 10μM, respectively. Specific BK and HIS receptor subtype analogues and antagonists revealed that PGE_2 release was mediated through B_2-type BK receptors and H_1-type HIS receptors. These receptor sub-types also mediate BK and HIS-induced phospholipase C activation. TPA, which directly activates protein kinase C, by itself did not stimulate PGE_2 release, but synergistically stimulated BK and HIS-induced PGE_2 release (4.5-fold), suggesting the involvement of protein kinase C in this response. Stimulation by BK, HIS and PAF of pro-inflmmatory eicosanoid release from keratinocytes may be an important mechanism through which these mediators induce cutaneous inflammation.

CHARACTERIZATION OF BULLOUS PEMPHIGOID ANTIBODIES BY USE OF RECOMBINANT BULLOUS PEMPHIGOID ANTIGEN PROTEIN. Masaru Tanaka, Takashi Hashimoto, Masayuki Amagai, Nobuyoshi Shimizu*, Takuichi Tsubata#, Akira Hasegawa#, Keizaburo Miki# and Takeji Nishikawa, Department of Dermatology and Molecular Biology*, Keio University School of medicine, Tokyo and Tonen Corporation#, Saitama, Japan

The purpose of this study is to characterize bullous pemphigoid (BP) antibodies using recombinant BP antigen protein. We previously obteined a mouse cDNA clone by use of human monoclonal anti-basement membrane zone antibody which exclusively reacted with a 230kD BP antigen. The cDNA was shown to encode for about half of the 230kD protein at C-terminus. We elaborated recombinant BP antigen protein by recloning the cDNA insert into plasmid vecter pUC9 and observed the reactivities against BP antibodies. Sera from 133 patients with BP, 21 normal volunteers and 20 patients with pemphigus were tested by Western blot technique using both extract of human epidermal and recombinant BP antigen protein. Two major protein bands of 230kD and 170kD in epidermal extract were detected by BP sera but not by any control or pemphigus sera. These 230kD and 170kD bands were recognized by 99(74%) and 68(51%) of 133 BP sera, respectively. Recombinant BP protein was 120kD and was recognized by 84(63%) BP sera, all of which detected 230kD band. All 34 BP sera showing only 170kD band or none of these bands, did not detect 120kD recombinant protein band. These results confirmed that 120kD recombinant protein is a part of the 230kD protein. Furthermore, these results suggested that 120kD protein does not share any of 170kD antigen epitopes, although we can not exclude a possibility that the 170kD protein is a part of the 230kD protein at N-terminus.

Differential effect of UVB irradiation and anti-Ia antibody plus complement treatment on epidermal cells for the induction of delayed-type hypersensitivity reaction and for cytotoxic T lymphocyte generation to alloantigens. Kunihiko Tamaki, Masafumi Fujima, Yamanashi Medical College, Showa University School of Medicine, Japan.

The role of epidermal Langerhans cells(LC) in alloimmunity is still a matter of controversy. In a previous paper we have shown that LC were required for both induction of DTH reaction and CTL response to alloantigens using UVB irradiation or by depletion of LC using anti-Ia antibody(Ab) plus complement(C). The present paper was conducted to compare the effect of UVB irradiation and anti-Ia Ab plus C treatment on alloimmunity. Epidermal cells(EC) were prepared from BALB/c skin transplanted onto $(C3HXBALB/c)F_1$ $(C3CF_1)$mice 150 days after skin graft. LC of parental skin transplanted on F_1mice have been shown to be totally repopulated by recipient LC by day 50. EC were injected subcutaneously into normal BALB/c mice after UVB irradiation or anti-Ia Ab plus C treatment. Seven days later, these mice were subjected to assay for DTH reaction or CTL activity. For DTH reaction, C3H spleen cells (SC) were injected into foot pad and foot pad swelling was measured 24 hours later. Significant reduction was noted in both groups of mice. For CTL activity, SC of these mice were assessed after in vitro stimulation. CTL activity in mice sensitized with EC after anti-Ia Ab plus C treatment showed appreciable reduction whereas that of after UVB irradiation did not. Thus, differential effect of UVB irradiation and anti-Ia plus C treatment was noticed in alloimmunity.

INDUCED-FORMATION OF A NOVEL POLYPHOSPHOINOSITIDE IN CULTURED HUMAN KERATINOCYTES BY LEUKOTRIENE B_4. Wilson Tang and Vincent A. Ziboh, Department of Dermatology, University of California, Davis, School of Medicine, Davis, CA.

Receptor-mediated hydrolysis of phosphatidyl 4,5-bisphosphate ($PtdIns4,5P_2$) has been implicated in the transduction of a variety of intracellular signals mediating cell functions. That growth promoting and mitogenic responses of Growth Factors involve the generation of novel polyphosphoinositides catalyzed by inositol phospholipid 3-kinase have recently been suggested. Since leukotriene B_4 (LTB_4) possesses a receptor on keratinocytes and is reported to enhance DNA synthesis in keratinocytes we tested its ability to modulate the inositol phospholipid metabolism in these cells. Specifically, normal human keratinocytes (NHEK from Clonetics, Inc.) were initially cultured at 37°C to 80% confluency in keratinocyte growth medium (KGM) supplemented with bovine pituitary extract, and 5% CO_2. The cells were labeled with ^{3}H-inositol (2.5μCi/ml) for 48 hrs. The labeled medium was siphoned off and the cells challenged with or without varying concentrations of LTB_4 (50-100nM) for 0,15,30 and 60 sec. respectively. Labeled lipids were extracted with acidified chloroform/methanol (2:1) and the inositol phospholipids separated by thin layer chromatography. LTB_4-induced novel radioactive peak was eluted, deacylated and deglycerated. Analysis of the resulting products by HPLC, revealed a radioactive peak that co-migrated with authentic standard of inositol 1,3,4,5-tetrakisphosphate, a deacylated and deglycerated product of phosphatidyl 3,4,5-trisphosphate ($PtdIns3,4,5P_3$). This finding indicates the presence of a phosphatidyl 4,5-bisphosphate ($PtdIns4,5P_2$) 3-kinase activity in LTB_4-treated human keratinocytes. This activity was negligible in non LTB_4-treated cells. The transient formation of this novel phospholipid by LTB_4-treated keratinocytes suggests that a possible alternative mechanism may exist for proliferative signals in human keratinocytes which may modulate cellular proliferation.

EFFECTS OF PROTEINASE INHIBITORS AND Ca^{2+} ANTAGONIST ON EXPERIMENTAL SUBGRANULAR EPIDERMOLYSIS CAUSED BY RECOMBINANT EPIDERMOLYTIC TOXIN A. Shigeru Taniguchi and Makoto Inaoki, Department of Dermatology, Kanazawa University School of Medicine, Kanazawa, Japan.

Epidermolytic toxin A (ETA) is one of the causative agents of staphylococcal scalded skin syndrome which is characterized by subgranular epidermolysis (SE). Though it has been reported that α_2-macroglobulin inhibited ETA activity, the mechanism of SE caused by ETA is still unclear. We investigated the pathogenic role of proteinase and Ca^{2+} in experimental SE using recombinant ETA. rETA was released from the periplasmic space of E. coli transformed by ETA gene by osmotic shock treatment and purified by HPLC. Small pieces of one-day-old mouse skin were cultured in MEM containing rETA. α_2-mactoglobulin, N-ethylmaleimide, leupeptin, E-64, PMSF, pepstatin, EDTA, EGTA and TMB-8 were added at various concentration. The epidermolytic activity of the purified rETA was 5,000 EU/mg. In the presence of 10 μg/ml rETA, SE occurred at 4 hours and was not inhibited by the proteinase inhibitors except EDTA and EGTA. EDTA, EGTA and TMB-8 inhibited SE completely at the concentration of 0.01-1mM, 0.1-1mM and 0.1-1mM respectively. However, these inhibitions were not observed after the addition of Ca^{2+} into medium. These results indicated that both Ca^{2+} influx and intracellular Ca^{2+} transport play a important role in SE caused by ETA.

MODULATION OF GAMMA-INTERFERON PRODUCTION BY RETINOIC ACID. Tadashi Terui, Barbara A. Araneo, Hyun Chul Lee, and Raymond A. Daynes, Department of Pathology, Univ. of Utah School of Medicine, Salt Lake City, Utah.

Retinoids have been used for the treatment of several kinds of immunologically-mediated dermatologic conditions. These include diseases such as psoriasis, lichen planus, and acne conglobata. In spite of the known effects of retinoids on epidermal keratinization, the mechanisms by which they derive their therapeutic benefit are not fully understood. We determined that the systemic administration of all-trans-retinoic acid reduced the ability of normal mice to elicit both delayed-type and contact-type hypersensitivity reactions. Since the elicitation of these cell-mediated immune responses is known to involve lymphokine secretion by activated $CD4^+$ T cells, we questioned whether retinoids were capable of modulating lymphokine production. Activated splenic lymphocytes obtained from retinoic acid-treated animals were found to secrete reduced levels of gamma-interferon (γIFN) and normal levels of IL-2 and IL-4 when compared to normal controls. This finding differed from the observed effects of glucocorticoids or $1,25(OH)_2D_3$, both of which caused a reduction in activated lymphocyte synthesis of γIFN and IL-2 while stimulating IL-4 production. A reduced capacity to produce γIFN was also found following a direct exposure of purified T cells or antigen-specific cloned T-cell lines to various doses of retinoic acid *in vitro* prior to their activation. Dehydroepiandrosterone, a natural androgen steroid hormone, could effectively reverse the inhibitory effects of retinoic acid on γIFN synthesis *in vitro* and *in vivo*. Our results suggest that retinoic acid may be exerting some of its therapeutic benefits via a capacity to regulate the production of γIFN by antigen-activated T cells.

EXPRESSION OF COMPLEMENT RECEPTORS IN THE EPIDERMIS. Francisco Tausk and Irma Gigli, Division of Dermatology, Univ. of California School of Medicine, San Diego, California.

To evaluate a possible complement regulatory function of the epidermis, we examined the expression of the C3b receptor (CR1), the decay accelerating factor (DAF) and the C3d/Epstein-Barr Virus Receptor (CR2) in fresh normal epidermis. The first two regulate the activation and function of the classical and alternative pathway convertases and mediate endocytosis. The latter binds C3d as well as EBV, resulting in the activation of B cells.

Utilizing monoclonal antibodies (25 ug/ml) and indirect immunofluorescence we evaluated the expression of these membrane proteins in adult normal skin and newborn foreskins. Staining with isotypic mouse IgG as well as anti-ICAM-1 at the same concentrations were consistently negative. CR1 was found exclusively in the basal layer of the epidermis of all the specimens studied. Fragments of C3 expressing the C3c epitope were observed in identical location. CR2 was found in a granular pattern on the membrane of cells in the upper two-thirds of the epidermis. DAF was present in normal basal membrane in addition to elastic fibers. In conclusion, the finding of CR1, CR2 and DAF in the epidermis suggests that: 1) Complement receptors may endow the epidermis, and keratinocytes in particular, with immune functions such as regulation of complement activation, and endocytosis of C3 opsonized particles. 2) Because of their identical location, C3c may be passively bound to keratinocytes expressing CR1. 3) The presence of CR2 may render keratinocytes susceptible to EBV infection, and 4) fragments of C3 may have trophic functions on keratinocytes.

Mechanism of Mast Cell Granule (MCG)-Mediated Cytotoxicity In Vitro. **MD Tharp, R Goldfarb, LJ Chan. Department of Dermatology and Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh PA.** Rat connective tissue mast cells (CTMC), like those found in the skin, can kill the tumor cell lines WEHI-164 and 5C25 in vitro by a TNF-like peptide and serine protease-mediated mechanism. Isolated MCG also are able to kill these tumor targets; however, their mechanism of action has not been defined. The purpose of this study was to investigate the mediator(s) of MCG killing of CTMC-sensitive targets. MCG were purified from a homogeneous population of rat CTMC. Granules were cultured at different whole CTMC effector to target (E:T) equivalent ratios with ^{51}Cr labeled tumor cells in the presence or absence of polyclonal anti-TNF antibodies (abs) and/or the protease inhibitor BBI (10μg/ml). Untreated MCG served as controls. In the presence of BBI, nearly a 40% reduction in MCG (E:T=40:1) killing of both WEHI-164 and 5C25 was observed when compared to MCG-mediated cytotoxicity. The addition of rabbit serum containing anti-TNF abs (2000 neutralizing units) plus BBI inhibited MCG killing of both targets by > 80% when compared to untreated MCG controls. The mechanism of MCG-mediated killing of the TNF-resistant target Currie also was investigated. BBI only slightly reduced MCG (E:T=40:1) lysis of Currie. While recombinant murine TNF failed to kill this target, serum containing anti-TNF abs significantly inhibited MCG-mediated killing. However, normal rabbit serum, devoid of anti-TNF abs, had a similar inhibitory effect. We conclude from these studies that MCG kill WEHI-164 and 5C25 by TNF-related and protease-mediated mechanisms, whereas killing of Currie involves a protease plus an additional non-TNF, serum-inhibitable mechanism.

MECHANISMS OF EPIDERMAL IMMUNOREACTIVITY IN ATOPIC DERMATITIS. R. Stan Taylor, Craig Hammerberg, Liza Chang, and Kevin D. Cooper, Immunodermatology Unit, Department of Dermatology, University of Michigan, Ann Arbor, Michigan.

The inflammatory dermatitis and cutaneous immunosuppression of atopic dermatitis (AD) may be related to the marked proliferation of resting AD T cells in response to autologous lesional epidermal cells (EC). The T cell activation is critically dependent upon interactions with class II MHC gene products expressed by $CD1^+DR^+$ Langerhans cells as well as $CD36(OKM5)^+DR^+$ cells. Since $CD36^+$ cells in UV-exposed skin and in blood preferentially activate a subset of T cells which respond to self antigens in the autologous mixed lymphocyte reaction (AMLR), we asked whether similar autoreactivity could be occurring in atopic skin. Indeed, lesional AD EC potently activated the AMLR-reactive T subset of $CD45R^+CD4^+$ T lymphocytes which induce maturation of suppressor cells (55285 cpm^3HTdR). However, $CD45R^-CD4^+$ T lymphocytes could also be activated (31572 cpm) suggesting that a population of recall-antigen-reactive helper cells may be recognizing antigens distinct from AMLR autoreactivity antigens. To address this question, we utilized bromodeoxyuridine (BUdR) plus light to lyse AD T cell clones reactive in the primary AMLR. Resting $CD4^+$ blood T cells from atopic patients were stimulated with autologous gamma irradiated blood antigen presenting cells to stimulate an AMLR (3582 cpm). On the second and third days of incubation, BUdR was added to the culture, and on the fourth day cells are exposed to 313 nm light. This treatment lysed clones responding in the AMLR (672 cpm), while sparing other clones with different antigenic reactivity (Tetanus Toxoid:14945 cpm). T cells depleted of AMLR responders were still able to proliferate to autologous EC (4727 cpm), but to a diminished level relative to nondepleted controls (6645 cpm). These findings show that T cells reactive to lesional AD EC contain autoreactive clones which respond to antigens presented in the AMLR, which may account for cutaneous immunosuppression. However, there also appear to be helper T cells which respond to other, possibly recall, antigens of either endogenous or exogenous origin, which may be of importance in the dermatitis and hyper IgE.

1,25-DIHYDROXYVITAMIN D_3: A NOVEL AGENT FOR WOUND HEALING. X.Q. Tian, T.C. Chen and M.F. Holick. Boston University School of Medicine, Boston, MA

1,25-Dihydroxyvitamin D_3 (1,25-D)has diverse effects in a variety of tissues and cell types including the stimulation of transglutaminase activity, fibronectin synthesis and cellular adhesion. These findings suggest a possible use of 1,25-D in wound healing. We investigated the effect of the topically applied 1,25-D or vehicle on the healing of cutaneous wounds (diameter:4mm, full thickness)in the rat in a double blinded manner. Wound areas were measured by planimetry technique. Healing was expressed as the percentage of the original wound area that was healed. 1,25-D at concentrations between 0.2 and 1.0 μg/day topically applied to the wound caused a dose-dependent acceleration of healing (%healing on day 4 expressed as means ± SEM: 19±4% for control; 32±3%, 34±5% and 37±4% for 1,25-D at concentrations of 0.2, 0.5 and 1.0 μg/day, respectively; 1,25-D vs. control $p<0.025$). Time course and specificity studies showed that 1,25-D specifically promoted wound healing between 2-5 days after wounding as compared with vitamin D at the dose of 1.0 μg/day(%healing on day 2, control: -2±7%, vitamin D_3: 0±9% and 1,25-D:16±6%; %healing on day 3 of the three groups: 9±7%; 7±9% and 27±7%; on day 4: 13±7%, 8±12% and 31±7%; day 5: 36±9%, 27±11% and 55±6%; 1,25-D vs. control: $p<0.05$; vitamin D_3 vs. control: no significant difference). Our results clearly demonstrate that 1,25-D significantly accelerates wound healing.

DIMINISHED CUTANEOUS HAIR DENSITY IN DYSPLASTIC NEVUS SYNDROME AND INVERSE RELATIONSHIP BETWEEN CUTANEOUS HAIR AND NEVI DENSITY. JK Tobacman, DC Whitaker, M Mori, C Kao. The University of Iowa College of Medicine, Iowa City, IA.

This investigation was undertaken to determine more information about cutaneous hair density in patients with dysplastic nevus syndrome. Clinical photographs of 41 subjects, 19 of whom are patients with dysplastic nevi, followed by the Department of Dermatology of the University of Iowa, and 22 of whom are age, race, male, aged 25-34, white, residents of Eastern Iowa, and of similar occupational, ethnic, social, and economic background. Terminal hair density was graded in all subjects at seven or more sites, using a modified version of a previously published grading system. Regions graded include: anterior chest, upper back, lower back, upper abdomen, lower abdomen, arm, forearm, thigh, and leg. In addition, nevi were counted in these regions in each subject. No data were tabulated, compared, or analyzed until all observations had been made. Terminal hair density was diminished in cases relative to controls (p-.007, student's t-test). In addition, linear regression analysis, demonstrated a significant inverse relationship between density of cutaneous hair and of nevi (p<.0001), occurring both in case and control subjects. These findings are consistent with the hypotheses that the melanocytes of the hair follicles may be the cells of origin of some of the pigmented lesions and that diminished cutaneous hair density may be a risk factor for development of some of the pigmented lesions, including dysplastic nevi.

SUPPRESSION OF GAMMA-INTERFERON INDUCED HLA-DR ANTIGEN EXPRESSION ON NORMAL AND TRANSFORMED KERATINOCYTES BY 1.25 $(OH)_2$VITAMIN D_3. Takeshi Tone, Hikaru Eto, Kensei Katsuoka, Kiyoshi Nishioka, Shigeo Nishiyama and Fumio Otani, Dept. of Dermatology and Transp. Immunology, Kitasato Univ. School of Medicine, Sagamihara, Japan.

Although it is known that the topical application of 1.25$(OH)_2$VitaminD_3(D3) is effective on psoriatic lesions, the mechanism is unclear. To elucidate the effect of VD3 on keratinocytes, we studied the effect of recombinant human gamma interferon(IFN-γ) and VD3 on the expression of HLA-DR and MHC class I antigen on 3 normal and 6 transformed keratinocytes by means of FACS analysis. IFN-γ (500JRU/ml,72hr) induced the expression of HLA-DR antigen on 3/3 normal keratinocytes, 3/4 trichilemmoma and 1/2 squamous cell carcinoma cell lines. When these cells were cultured with both IFN-γ and VD3 at the same time, the expression of HLA-DR antigen was significantly decreased. Sequential treatment of these cells with IFN-γ and VD3(each 72hr), or vice versa, showed significant suppression of HLA-DR expression. The mean fluorescence intensity of class I antigen was also decreased after VD3 treatment. In addition, the level of class I and II mRNA was studied by Northern and dot blot analysis. These results suggested that the effect of VD3 on psoriasis may be related to down regulation of HLA-DR expression on keratinocytes.

FUNCTIONAL CHARACTERIZATION OF A TUMORIGENIC MURINE VASCULAR ENDOTHELIAL CELL LINE (F-2). Ken-Ichi Toda, Yukiya Maruguchi, Kaoru Tsujioka, Yoshiki Miyachi, and Sadao Imamura, Dept. of Dermatol., Kyoto Univ., Fac. of Med., Kyoto, Japan.

Recently, we have reported a new established tumorigenic murine cell line from a ultraviolet light (UVL) induced tumor, which generates hemangiomatous lesions in nude mice and preserves morphological properties of vascular endothelial cells (EC) in in-vitro systems (J.I.D.92:534A 1989). In this study, we have further characterized functional properties of F-2 cells from both standpoints of EC and tumor cells. As an important functional marker of EC, we tested anti-coagulant activity of F-2 cells. Ionophore A23187 or tumor necrosis factor stimulated cultured F-2 cells with resultant PGI_2 synthesis, whereas they did not stimulate fibrosarcoma cell lines ∂1d or ♀1, which were also established from the UVL induced tumor. Furthermore, the culture media conditioned with F-2 cells showed marked anti-coagulant activity of murine or human whole blood at the concentration of 10% total volume, but that with fibrosarcoma cell line did not. Cell adherence studies, where cells were incubated on cell adhesion ligands-coated dishes in serum free medium for 45 min., showed that F-2 cells were as highly adhesive to fibronectin, type I collagen or basement membrane as normal human EC were, while ∂1d or ♀1 were less adhesive to these ligands. As tumor cell activities, we examined cell growth in soft agar, and angiogenesis. F-2 cells did not show contact inhibition in culture and were able to grow in soft agar to make small colonies. Culture supernatants of F-2 or fibrosarcoma cells showed enhanced tritiated thymidine incorporation into human EC. In vivo neovasculization as quantitated by the proliferation of corneal new blood vessels in rabbit was also observed. There were no significant differences of angiogenesis activities among those cell lines. Taken altogether, it can be concluded that F-2 is a unique tumor cell line which well preserves not only morphological but functional properties of vascular EC and may serve as a useful source for understanding EC biology.

INHIBITORS OF CALCIUM MOBILIZATION ARE POTENTIAL INHIBITORS OF EPIDERMAL DIFFERENTIATION. Philip Tong, Gary Lee, Gerald DeVries, Elizabeth Syage, Roshantha Chandraratna, Michael Garst and Larry Wheeler, Allergan Inc./Herbert Labs, Irvine, CA.

Manoalide (MLD), a sesterterpenoid, and some of its synthetic analogs are potent inhibitors of hormone- and voltage-induced calcium mobilization in a number of cell lines. Since calcium is a major mediator of epidermal differentiation, we have studied the effects of these compounds on epidermal differentiation by assaying cornified envelope (CE) formation in human epidermal keratinocyte cultures (KC). Confluent KC were pre-treated with various compounds for 24 hrs in 0.15 mM Ca^{++} basal MCDB 153 medium and induced to form CE by 1.4 mM Ca^{++} and 10^{-7}M phorbol ester (TPA) in the same medium for another 24 hrs. Untreated cultures formed 20-50% detergent-resistant CE in the assays. To benchmark the system, the IC_{50}'s for inhibition of CE formation by trans retinoic acid, AGN 190168 (retinoid), staurosporine (protein kinase C inhibitor), and lanthanum chloride (calcium channel blocker), were determined to be 0.0002, 0.0008, 0.008, and <0.1 uM, respectively. MLD, AGN 190383, and AGN 190742 inhibited thyroid hormone-induced and K^+-induced Ca^{++} signals with IC_{50}'s of 0.6/0.8, 0.6/1.0, and 3.5/1.5 uM, respectively, and inhibited CE formation by 50% at 0.1, 1, and 1 uM, respectively. AGN 190576, a selective phospholipase A_2 inhibitor, but inactive in Ca^{++} mobilization assays, did not inhibit CE formation. The results suggest that inhibitors of Ca^{++} entry and mobilization could inhibit epidermal differentiation.

RELATIONSHIP BETWEEN THE NUMBER OF 8-MOP PHOTOADDUCTS AND BIOLOGICAL ACTIVITY IN MURINE KERATINOCYTES. Y.Tokura, F.Gasparro, and R.Edelson. Deptartment of Dermatology, Yale U., New Haven, CT.

To correlate 8-methoxypsoralen photoadduct formation with keratinocyte biological activity, single cell suspensions of epidermal cells from BALB/c mice were treated with 8-MOP and UVA. Greater doses of 8-MOP and UVA led to proportionately greater numbers of photoadducts. A dose reciprocity relationship existed between the amounts of 8-MOP and UVA. For example, 0.9 adducts/million bases formed when cells were treated with either 10 ng/ml 8-MOP and 3 J/cm^2 or 15 ng/ml and 2 J/cm^2. Inhibition of [^{3}H]TdR incorporation and reduction of viability were observed in cells containing more than 1 adduct/million bases; 50% suppression of [^{3}H]TdR incorporation occurred at 5 adducts/million bases. However, fewer adducts were required to affect ETAF/IL-1 production. Cells with 2.5 adducts/million bases exhibited 50% suppression of ETAF activity in the cell-associated form. For cells treated with low doses of 8-MOP and UVA, 1-day culture with DMEM containing 10% FBS led to the removal of 51% of the photoadducts. However, culture studies using keratinocyte growth medium containing various concentrations of calcium (0.15-1.80 mM) showed that the removal depended on the calcium concentration. Photoadduct repair was associated with the recovery of ETAF/IL-1 production. Thus, these studies demonstrated that the formation and removal of 8-MOP photoadducts was closely related to the levels of keratinocyte biological activity.

STAUROSPORINE, A PROTEIN KINASE C INHIBITOR, PREVENTS DECREASED PROLIFERATION IN KERATINOCYTE CULTURES CAUSED BY HIGH LEVELS OF MITOGENS. Philip S. Tong, Vincent M. Lee and Larry A. Wheeler, Dept. of Biological Sciences, Allergan, Inc./Herbert Labs, Irvine, CA

In psoriasis, protein kinase C (PKC) activity in the epidermis has been reported to be lower than in normal epidermis. To investigate if decreased PKC activity plays a role in epidermal hyperproliferation, we have studied the effect of a potent PKC inhibitor, staurosporine (STAU), on human epidermal keratinocyte (KC) proliferation. Foreskin KC were stimulated to synthesize DNA by transforming growth factor alpha (TGF_α) or epidermal growth factor (EGF) in basal medium in the presence or absence of STAU for 24 hrs, followed by a 2-hr labeling with ^{3}H-thymidine. The results (cpm/µg DNA) show that (1) both growth factors induced a dose-related increase in DNA synthesis with optimal stimulation between 1 and 3 ng/ml. At 10 to 100 ng/ml, DNA synthesis decreased. (2) The presence of 10^{-13} to 10^{-10}M STAU maintained the stimulation by ≥ 10 ng/ml of growth factors at high levels. (3) Anti-bromodeoxyuridine histochemical technique showed that 10^{-13} and 10^{-12}M STAU increased labeled nuclei by 18.1% and 58.8%, respectively, over cultures stimulated by 100 ng/ml TGF_α alone. (4) STAU alone (≤ 10^{-10}M) caused no change or a slight stimulation of DNA synthesis and was not toxic to KC as assayed by fluorescein diacetate uptake. The results suggest that (1) high levels of TGF_α or EGF will cause feedback regulation of KC proliferation, and (2) inhibition of PKC in KC or decreased PKC activity in psoriatic epidermis could prevent such regulation and could thus lead to hyperproliferation.

INTEGRIN MATRIX RECEPTOR EXPRESSION IN THE BLOOD VESSELS OF HEALING CUTANEOUS WOUNDS. Marcia G. Tonnesen, James Gailit, Erkki Ruoslahti, Richard A.F. Clark, National Jewish Center, Denver, CO; La Jolla Cancer Research Foundation, LaJolla, CA

Angiogenesis is crucial for new tissue formation during cutaneous wound healing. Neovascular cells, surrounded first by a provisional matrix and later by a basement membrane, presumably interact with these substrata through specific extracellular matrix (ECM) receptors. Integrin αβ heterodimeric transmembrane glycoproteins containing a β1 or a β3 subunit combined with a specific α subunit form such ECM receptors. Porcine cutaneous wound repair was chosen as a model for the investigation of angiogenesis, and microvascular maturation, organization, and subsequent regression. Immunofluoresence probing was performed at 5, 7, 10, and 14 days after full thickness wounding. Anti-laminin was used to delineate vascular integrity and maturation. Rabbit polyclonal antibodies specific for the fibronectin receptor (α5β1) or vitronectin receptor (αvβ3) integrin subunits were used to examine ECM receptor expression. In nascent vasculature of 5 day wounds, laminin appeared as diffuse granules in cells and as a broken stitch-work in the basement membrane zone (BMZ). By 7 days laminin formed bright continuous lines in the BMZ which began to fade in 10 and 14 day regressing vessels. In 5 day wounds fibronectin and vitronectin receptors appeared as diffuse granules in neovascular cells; at 7 days, as bright continous lines along the BMZ; at 10 days, as faded broken stitch-work along the BMZ; at 14 days, as dim granules when present. Thus integrin β1 and β3 ECM receptors appear to play a dynamic role in neovascularization.

PSORIASIS: AN IMMUNOHISTOCHEMICAL STUDY. Jaime A. Tschen, Amy B. Koff, and Annetta Walker, Departments of Dermatology and Pathology, Baylor College of Medicine, Houston, TX.

Recent studies suggest that the dermis has a prominent role in the epidermal proliferation of psoriasis. Most notably, psoriatic fibroblasts have been shown to induce the proliferation of normal keratinocytes in vitro.

The purpose of this study was to evaluate a quantitative difference in dermal fibroblasts when compared with uninvolved skin. Lesional and uninvolved skin were compared with skin from a control patient with no evidence of psoriasis. We demonstrate increased numbers of fibroblasts in lesional psoriatic dermis utilizing vimentin and factor XIIIa immunoperoxidase stains. This study complements in vitro models of psoriasis.

Langerhans cells, blood vessels, melanocytes, sweat glands, keratinocytes, and basement membranes were also compared in lesional and uninvolved psoriatic skin using appropriate histochemical and immunohistochemical stains. Our findings confirmed the observation that lesional psoriatic skin demonstrates fewer numbers of Langerhans cells with aconcentration of cells in the dermal papillae compared with the distribution observed in normal skin. Prominent vascular proliferations in psoriatic lesions were observed in sections stained with Eulex europaeus, vimentin, and factor XIIIa.

CORRELATION OF DIRECT IMMUNOFLUORESCENT (IF) FINDINGS IN BULLOUS PEMPHIGOID (BP) CASES TO DISEASE ACTIVITY. JD Torzecka*, A Lotowiecka-Wranicz*, A Sysa-Jedrzejowska*, E Waszczykowska*, B Dziankowska-Bartkowiak*, TP Chorzelski**, V Kumar***, EH Beutner***, *Dept. of Dermatol., Univ. of Ludz, Poland; **Dept. of Dermatol., Warsaw Acad. of Medicine, Warsaw, Poland; *** Dept. of Microbiol. and Dermatol., Univ. at Buffalo, SUNY, USA

In BP, basement membrane zone (BMZ) antibodies as seen by indirect IF studies sometimes relate to disease activity, but not reliably so. For example, 2 sera were positive in 16 BP cases in complete remission. We asked if direct IF reactions can provide prognostic information. All of 35 newly diagnosed BP cases were positive for BMZ deposits: 24 for complement (C3) and IgG, 4 for C3 and 7 for IgG. During treatment of 19 (of 35) 13 were direct IF positive, 6 for IgG and C3, 1 for C3 and 6 for IgG deposits in the BMZ. When treatment of 16 of the 35 BP cases was ended, 6 were direct IF positive, 4 for IgG alone and 2 for IgG and C3; 2 of these 6 relapsed. These findings show that: 1) direct IF demonstration of C3 in the normal skin of the wrist affords a more reliable marker of disease activity than indirect IF and 2) negative direct IFfindings in the BMZ of the skin from the wrist of BP cases can afford a guide to when treatment can be discontinued.

INVERSE RELATIONSHIP BETWEEN INVASION OF THE AMNIOTIC MEMBRANE AND PLASMINOGEN ACTIVATOR ACTIVITY. Ryoji Tsuboi, Hideoki Ogawa, and Daniel B. Rifkin*. Department of Dermatology, Juntendo Univ. School of Medicine, Tokyo, *Department of Cell Biology, New York Univ. Medical Center, New York, NY

Three human tumor cell lines, Bowes melanoma, HT1080 and Osmond cells, were characterized with respect to their ability to invade the amniotic membrane as well as protease levels. Bowes melanoma cells, which release a large amount of tPA, were poorly invasive on the amniotic membrane. Interestingly, the addition of plasmin inhibitors, or anti-tPA antibody enhanced invasiveness. Similarly, HT1080 cells, which produce very high levels of uPA, were also poorly invasive, but invasion was enhanced by plasmin inhibitors. Conversely, Osmond cells, which produce very low levels of PA, were very invasive on the amniotic membrane. The invasiveness of these cells was blocked by plasmin inhibitors.

These results suggest that invasion might require an optimal level of PA activity. High levels of plasmin, generated by tPA or uPA secreted by the cells, may cause uncontrolled matrix degradation and interrupt the interaction of cells and matrix in the early stage of invasion. The inhibition of excessive plasmin activity may stabilize and increase cell matrix contacts and result in an enhancement of invasiveness.

THE ABILITY OF NEUTROPHILS TO RELEASE OXYGEN FREE RADICALS IS REDUCED BY EXTRACORPOREAL PHOTOCHEMOTHERAPY (PHOTOPHERESIS). F.Trautinger[1], R.M.Knobler[1], W.Granninger[2], W.Macheiner[2], R.Neumann[1], M.Micksche[3], 1)II.Dept.of Dermatology, 2)II.Dept.of Int.Med., 3)Inst.for Appl.and Exp.Oncology, Univ.of Vienna, AUSTRIA

Neutrophil respiratory burst activity (RBA) was investigated in 8 patients (pts: 4 with LE, 2 with PSS, 2 with CTCL), who where treated with photopheresis (PP). Patient's buffy coat was obtained before and after PP. Prior to stimulation of neutrophil-NADPH oxidase with PMA (40nM) peripheral blood neutrophils were isolated by dextran sedimentation, ficoll density gradient centrifugation and hypotonic shock. Respiratory burst activity (RBA) was evaluated using the method of chemiluminescence with the help of a luminometer. The amount of O_2^- and H_2O_2 released during RBA was determined with the cytochrom-c and phenolred assays. Similarly neutrophils of healthy donors were evaluated after in vitro incubation for 20 mts. with different concentrations of 8-MOP and 5-MOP (100ng/ml, 50ng/ml, 25ng/ml, 0 [control]) before and after UV-A radiation.

A significant decrease of neutrophil RBA (up to 50%) after UV-A radiation was found in all patients. In vitro inhibition of RBA was directly correlated to 8-MOP and 5-MOP concentrations as well as UV-A dosage. Neither Psoralens alone nor UV-A alone (at 2 J/cm²) affected neutrophil RBA.

Our data suggest that during PP neutrophil NADPH activity, which is responsible for the release of tissue damaging oxygen free radicals, is specifically inhibited.

INTRODUCTION OF v-ras^{Ha} SUPPRESSES MELANOGENESIS IN MURINE MELANOCYTES. Katsuhiko Tsukamoto, Masato Ueda, Wilfred Vieira, and Vincent Hearing, Laboratory of Cell Biology and Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

The family of ras genes encodes a highly conserved protein (P21) that can influence cell growth and differentiation through its role in signal transduction pathways. The relationship between the ras gene and melanocyte differentiation (pigmentation) has not yet been clarified. The v-ras^{Ha} gene was introduced into immortal murine melanocytes, termed melan-a (Int J Canc 39:414,1987), using a defective retroviral vector. Infected cells (melan-a-ras) were shown by Northern hybridization to express the exogenous v-ras^{Ha} gene. The melan-a-ras cells lost their pigmentation in vitro and were growth suppressed by 12-o-tetradecanoyl-phorbol-13-acetate (TPA), which is necessary for the proliferation of the parental melan-a cells. The melan-a-ras cells were subcutaneously injected into nude mice and amelanotic melanomas were obtained, although the parental melan-a cells were not tumorigenic. Tyrosinase activity in melan-a and melan-a-ras cells was measured by the ^{3}H-tyrosine hydroxylase and ^{14}C-melanin synthesis assays and, although quite high in melan-a cells, was almost completely lost in melan-a-ras cells. Although tyrosinase activity in melan-a cells was increased by the treatment with α-MSH ($2x10^{-7}$M) in vitro, that of melan-a-ras cells was not altered. Immunoprecipitation using tyrosinase specific antibodies (αPEP1, αPEP7) failed to detect the synthesis of tyrosinase protein in melan-a-ras cells. The exact mechanisms involved in the suppression of melanogenesis by the ras oncogene is currently being actively investigated.

SOLUBLE SUPPRESSOR FACTORS PRODUCED BY PERIPHERAL BLOOD MONONUCLEAR CELLS DURING PAPILLOMA VIRUS INFECTIONS. S. Tyring and S. Baron, Departments of Microbiology and Dermatology, Univ. of Texas Medical Branch, Galveston, TX.

Patients with severe papilloma virus infections presenting as condyloma acuminatum (CA) or epidermodysplasia verruciformis (EV) often have depressed cell mediated immunity. Peripheral blood mononuclear cells (PBMC) from CA and EV patients when cultured for 3-5 days, released a soluble factor into the media that suppressed phytohemagglutinin (PHA)-driven proliferation and IL-2 production by lymphocytes from healthy controls. This soluble suppressor factor (SSF) was absent in healthy controls and was absent or present only in low concentrations in culture supernatants from CA patients who had been successfully treated. This SSF in various dilutions also prevented the proliferation of CTLL-2 cells in the presence of natural IL-2, measured by ^{3}H-thymidine uptake. The SSF had a molecular weight greater than 14,000, was heat labile, and acid labile at pH 2.0. Similarly, a soluble suppressor factor was released in the supernatants of PBMC from New Zealand white rabbits infected with Shope papilloma virus. This suppressor factor was also capable of aborting in vitro IL-2 production and proliferation of normal rabbit PBMC in response to PHA and the in vitro proliferation of CTLL-2 cells in an IL-2 dependent assay. This inhibitor of IL-2 production/activity may block T cell function in vivo and thus contribute to depressed cellular immunity in patients with severe papilloma virus infections.

EFFECTS OF PSK ON THE TUMOR FORMATION BY CHEMICAL CARCINOGENESIS IN MOUSE SKIN. Atsumichi Urabe#, Juichiro Nakayama#, Doo Chan Moon##, Hiroshi Terao#, Hiroo Kinoshita### and Yoshiaki Hori#,#Dept. of Dermatology, Faculty of Medicine, Kyushu University, ##Dept. of Dermatology, College of Medicine, Pusan National University, ###Dept. of Chemistry, School of Medical Technology, Kyushu University, Fukuoka, Japan.

PSK, a protein-bound polysaccharide derived from coriolus vesicolor, Quel. of basidiomycetes, is known to have an antitumor activity mediat ed by their ability to stimulate host immune defense mechanisms. Several experimental studies concerning on the anti-tumor effects with the established cancer cells have been performed, but the anti-carcino genic effect of this drug is unclear. In this study, we investigated the effect of PSK on the tumorigenesis in mouse skin (DDD mouse strain, female) either initiated by benzo(a)pyrene (B(a)P) and promoted by 12-O-tetradecanoyl-phorbol-13-acetate (TPA)(Group I) or initiated and promoted by B(a)P (Group II). Twelve mice of each group were fed with or without PSK contained alimentation (4%). Until the end of the experi ment (28 weeks), two carcinoma-baden mice were dead in Group I mice fed without PSK (PSK(-)), however in the mice fed with PSK in Group I (PSK (+)), any carcinoma was not identified at all, although considerable number of papilloma developed. In Group II mice, carcinomas started to be evolved at the 15th week of the experiment being irrelevant to PSK treatment. However, the number of carcinoma observed in the PSK(+) mice of Group II was clearly decreased in comparison with that in the PSK(-) ones.

SUPPRESSION OF GRAFT V. HOST DISEASE BY PSORALEN PLUS UVA THERAPY. Stephen E. Ullrich, Dept. of Immunology, M.D. Anderson Cancer Center, Houston, Texas.

Bone marrow transplantation is an effective treatment for a variety of hematological disorders. A major complication arising from bone marrow transplantation is the development of graft v. host disease (GVHD). Although depleting T cells from the bone marrow inoculum prevents the induction of GVHD, T cell depletion increases the probability of graft failure. The approach taken in this study was to inactivate rather than deplete alloreactive T cells within the bone marrow inoculum with psoralen plus UVA (PUVA) therapy and examine the effect this conditioning protocol had on the induction of GVHD. Donor C3H/HeN mice were injected with 0.4 mg of 8-methoxypsoralen and two hours later their spleen and bone marrow cells were removed and exposed in vitro to 560 J/m^2 of UVA radiation. PUVA treatment suppressed the ability of donor T cells to respond to alloantigen in the in vitro mixed lymphocyte culture. PUVA-treatment did not compromise stem cell viability or function as measured by a stem cell colony forming assay. The PUVA-treated cells were then used to reconstitute lethally x-irradiated (900 rads) BALB/c mice. Transfer of normal spleen and bone marrow cells into x-irradiated mice resulted in acute lethal GVHD, with a median survival time (MST) of 10 days. When PUVA-treated cells were used to reconstitute the lethally irradiated recipient mice, a significant prolongation of survival was observed (MST 44 days). Treatment of the donor cells with 8-methoxypsoralen alone or UVA alone did not significantly suppress the induction of GVHD. Examination of the haplotype of the peripheral blood leukocytes of the long-term survivors indicated that 70-80% of the cells were of donor origin, indicating engraftment. These findings demonstrate that psoralen plus UVA therapy can be used to condition donor bone marrow cells prior to bone marrow transplantation and suppress the induction of GVHD.

ULTRAVIOLET LIGHT INDUCES INCREASED CIRCULATING INTERLEUKIN 6 LEVELS IN HUMANS. Agatha Urbanski*, Thomas Schwarz", Peter Neuner*, Andreas Köck*, Jean Krutmann*, Reinhard Kirnbauer*, Thomas A.Luger*·", *Dept.Derm.II and LBI-DVS, Lab.Cellbiol., Univ.Vienna; "Dept.Derm., Hospital Vienna-Lainz, Austria; "Dept.Derm., Univ.Münster, FRG

Severe sun exposure can induce fever, leukocytosis and increased release of acute phase proteins e.g. CRP. The mechanisms mediating these systemic effects are only poorly understood. Interleukin 6 (IL 6) is a multifunctional cytokine causing fever and acute phase response which recently has been demonstrated to be produced by keratinocytes. The present study was performed to investigate the effect of a single UVB dose eliciting severe sunburn reaction on the production of IL 6 in vivo. Six human volunteers were treated with a single total body UVB-exposure equivalent to 4 minimal erythema doses. Plasma obtained at different time points before and after UV-irradiation was tested for IL 6 activity in the B9 assay. In contrast to plasma obtained before UV-exposure, post-UV specimens contained significantly elevated IL 6 levels peaking after 12 hr. Plasma IL 6 activity could be neutralized by an antiserum directed against recombinant human IL 6 and HPLC gel filtration revealed a m.w. of approximately 20 kD for plasma IL 6. Moreover, plasma IL 6 levels correlated remarkably with clinical symptoms and fever course peaking at 12 hr followed by an increase of serum CRP. These data indicate that IL 6 which is released by keratinocytes upon UV-irradiation, may gain access to the circulation and via its pyrogenic as well as acute phase inducing effects may function as an important mediator in the pathogenesis of severe sunburn reactions.

ANDROGEN-DEPENDENT ACTIVITY OF THE SEBACEOUS GLAND AND THE EFFECT OF TOPICAL TRETINOIN IN FUZZY RATS. H. Uno, T.D. Fors, S.M. Packard, G.S. Bazzano, and A.M. Kligman, Primate Res. Ctr., Univ. of Wisconsin-Madison; Sch. of Public Health, Tulane Univ., New Orleans; and Dept. of Dermatology, Univ. of Pennsylvania, Philadelphia.

Fuzzy rats, a genetic mutant between hairless and haired albino rats (Ferguson et al., 1979), exhibit androgen-dependent sebum production and enlargement of the sebaceous glands. A thick coating of sebum on fuzzy-haired skin is characteristic of postpubertal males (60 days old, serum level of testosterone [T] 2 to 5 ng/ml). Sebum production was markedly suppressed after castration and reactivated by either a T or a dihydrotestosterone implant, but not by estrogen or progesterone. Similar responses were found in ovariectomized rats after implants of the steroids. The rate of sebum secretion measured by Sebotape, and micro-morphometry of the size of sebaceous lobules yielded data in close agreement with the gross appearance of sebum secretion. Using 30 rats, 10 prepubertal and 20 adult males, tretinoin (0.05%, Ortho Corp.) or vehicle was applied topically on the back for 2 months. After one month the tretinoin group showed a marked reduction of sebum secretion. At two months the epidermis showed a minimum degree of hyperkeratosis and acanthosis, but the dermis showed no changes. The size of sebaceous lobules stained with Sudan Black B in serial frozen sections (30 μ) and digitized by computer was reduced 20% compared to controls. The size of individual sebocytes showed no difference between the two groups. Tretinoin appeared to suppress the rate of cell turnover of the sebocytes, reducing sebum secretion in hyperplastic sebaceous glands.

LABORATORY DIFFERENTIATION OF BULLOUS PEMPHIGOID FROM EPIDERMOLYSIS BULLOSA ACQUISITA ON PREVIOUSLY FROZEN SKIN BIOPSIES AND SUBSEQUENTLY SPLIT IN NaCl. J.E. Valeski, V. Kumar, E.H. Beutner, C. Cartone, and K. Kasprzyk, IMMCO Diagnostics, Inc., Buffalo, NY and Department of Department of Microbiology, State University of New York at Buffalo

Often patients with Bullous Pemphigoid (BP) and Epidermolysis Bullous Acquisita (EBA) have negative serum basement membrane zone (BMZ) antibodies while having linear BMZ staining of skin biopsies by routine direct immunofluorescence (IF). IF mapping studies for collagen IV and laminin may yield misleading results. The only other reported way to differentiate BP from EBA is to rebiopsy normal skin, split the biopsy in NaCl and examine the location of IgG at the split site by direct IF. We investigated the utility of splitting in NaCl previously frozen normal skin biopsies from 8 cases of BP and 3 EBA. All cases were characterized by serum studies on normal split skin and/or by antigen mapping on patient lesional skin biopsy. Frozen skin biopsies were placed in 1M NaCl for approximately 48 hours. Frozen sections were cut at 4 u and examined by direct IF. 10/11 biopsies revealed the split at the proper site, i.e. 3/3 EBA biopsies showed IgG at the floor of the blister. 7/7 BP biopsies showed staining at the roof only (6/7) or both the roof and floor (1/7), confirming the diagnosis of EBA and BP, respectively. The splitting of previously frozen normal skin biopsies may be used for the differential diagnosis of BP from EBA, and in most cases may eliminate the inconvenience, expense and diagnostic delay of a repeat biopsy.

VARIATION IN UTILIZATION OF INPATIENT HOSPITALIZATION FOR TREATMENT OF PSORIASIS: ONE HOSPITAL'S EXPERIENCE. Abby S. Van Voorhees and John S. Hughes, Division of Dermatology, UConn Health Center, Farmington, CT, and Department of Internal Medicine, Yale Univ. School of Medicine, New Haven, CT.

Over the past ten years, a number of factors, both medical as well as social, have contributed to altered utilization of inpatient resources. The impact of these changes on dermatologic care are largely unexplored. Therefore, we have analyzed the rate of hospitalization for psoriasis (ICD-9 Code # 696.1) over a ten year period in one hospital. Length of stay declined from 1980 through 1989 by 37%. Number of total admissions for this ICD-9 code also declined during this interval. These declines were seen in both psoriasis patients on the dermatologic service as well as those on other services. The greatest period of decline coincided with the implementation of the Prospective Payment system (1983-1984). These data suggest that changes in therapeutic modalities and reimbursement schemes have resulted in modification of the approach to psoriasis.

ULTRAVIOLET B AND TNF-ALPHA CAUSE SIMILAR ALTERATIONS IN INTRAEPIDERMAL LANGERHANS CELLS. M. H. Vermeer and J. W. Streilein. Department of Microbiology and Immunology, Unversity of Miami School of Medicine, Miami, Florida.

Acute, low dose ultraviolet B radiation (UVB) of mouse skin disrupts the normal morphology of epidermal Langerhans cells (LC) and, in some strains of mice, impairs the capacity of skin to sustain the induction of contact hypersensitivity (CH). It was recently demonstrated that tumor necrosis factor-alpha (TNF_a) also impairs the induction of CH if hapten is painted on skin in which TNF_a was injected intradermally (ID). To determine whether TNF_a might have a similarly deleterious effect on LC, we have compared the effects of UVB and of TNF_a on the density and morphology of murine LC. Epidermal sheets were prepared 2 hours after shaved, body wall skin of BALB/c male mice had been treated with a single exposure of UVB (400 J/m^2) or by a single ID injection of TNF_a (5 ug). LC density and morphology were evaluated microscopically after staining with anti-Ia^d, and with the aid of ADPase histochemistry. When compared to epidermis into which saline had been injected ID (control), TNF_a-treated epidermis contained fewer (<50%) identifiable LC; $ADPase^+$ cells that persisted were rounded, less intensely stained, and displayed few dendrites. Similar changes in number and morphology of LC were observed in UVB-treated epidermis. These findings indicate that epidermal LC are altered morphologically by exposure to TNF_a in situ. Since similar effects are observed after UVB treatment, and since both TNF_a and UVB can impair CH induction, we propose that TNF_a may inhibit antigen presentation by LC and that this cytokine may be an important mediator of the deleterious effects of UVB on cutaneous immune responses.

RETINOIC ACID STIMULATION OF HUMAN DERMAL FIBROBLAST PROLIFERATION IS ASSOCIATED WITH MOBILIZATION OF INTRACELLULAR Ca^{2+}. James Varani, Jay Shayevitz, Debra Perry, Raj S. Mitra, Brian J. Nickoloff and John J. Voorhees. Departments of Pathology, Anesthesiology and Dermatology, The University of Michigan Medical School, Ann Arbor, MI.

Human dermal fibroblasts failed to proliferate when cultured in medium containing 0.15 mM Ca^{2+} but did when the external Ca^{2+} concentration was raised to 1.4 mM. All-trans retinoic acid (retinoic acid) stimulated proliferation in low-Ca^{2+} medium but did not further stimulate growth in Ca^{2+}-supplemented medium. Measurement of intracellular Ca 2+ concentrations with the fluorescent dye Fura-2 indicated that the intracellular free Ca^{2+} concentration of cells grown in low-Ca^{2+}, medium was approximately 30 nM and in high-Ca^{2+} medium was 83 nM. Treatment of fibroblasts in low Ca^{2+} medium with retinoic acid led to an increase in the intracellular free Ca^{2+} level which was similar to the level obtained in cells cultured in Ca^{2+}-supplemented medium. Using $^{45}Ca^{2+}$ inhibited release of intracellular Ca^{2+} into the extracellular fluid. Retinoic acid also stimulated ^{35}S-methionine incorporation into trichloroacetic acid-precipitable material but in contrast to its effect on proliferation, stimulation of ^{35}S-methionine incorporation into trichloroacetic acid precipitable material but in contrast to its effect on proliferation, stimulation of ^{35}S-methionine incorporation occurred in both high-Ca^{2+} and low Ca^{2+} medium. These data indicate that retinoic acid stimulation of proliferation, but not protein synthesis, is associated with mobilization of intracellular Ca^{2+} and inhibition of Ca^{2+} egress.

TRANSDERMAL DELIVERY OF A NOVEL 5-LOI. I. FLUX AND METABOLISM. E. Wakshull and C. Desbonnet. Pfizer Central Research, Groton, Ct.

Experimental and clinical observations over the last few years have suggested that an agent with 5-lipoxygenase inhibitory (5-LOI) activity might be efficacious in the treatemnt of psoriasis. With the idea that topical treatment might obviate potentially undesirable systemic effects, we studied the topical delivery of a novel hydroxypyrimidine derivative (CP-70,490) with potent 5-LOI activity. The flux and metabolism of ^3H-CP-70,490 through both hairless mouse skin and human skin were determined using standard side-by-side diffusion cells. ^3H-CP-70,490 in 50% EtOH/H_2O was applied to the stratum corneum side, while the dermis side was exposed to Sorensen's buffer (receiver fluid) for up to 48 hrs. The results show that CP-70,490 had extremely low flux through both species. Values of $1\text{-}3\times10^{-11}$ moles/cm^2/hr (drug concentration 50ug/ml) were found in both mouse and human skin. HPLC analysis of receiver fluid revealed essentially no intact drug had crossed the mouse skin, while a small percentage of receiver fluid radioactivity from human skin samples represented intact drug. On the other hand, drug extracted from the skin was found to be completely intact. The estimated concentration of drug within the skin ranged from 166uM (human) to 1mM (mouse), fully 2-3 orders of magnitude greater than the in vitro IC_{50} for 5-LOI (0.3uM). These results suggest that CP-70,490, when applied topically, can reach pharmacologically relevant concentrations with the skin, while at the same time minimizing systemic exposure because of the combined effects of low flux and extensive dermal metabolism.

COMPOSITE GRAFTING OF CHRONIC LEG ULCERS WITH CULTURED AUTOLOGOUS DERMAL FIBROBLASTS AND EPIDERMAL CELLS. M. Varghese, DM Carter, M. Eisinger. The Rockefeller Univ. and Memorial Sloan Kettering Cancer Center, New York, N.Y.

Cultured autologous epidermal cell grafts have been used in the past for covering burn wounds. This technique has also been utilized in grafting leg ulcers that failed to heal by conventional means of treatment. Dermal tissue, an integral component of skin, provides an optimal substrate for epidermal graft. We therefore provided a dermal substitute at first, followed by epidermal cell grafts. Fibroblasts from a punch biopsy and epidermal cells from a suction blister roof were cultivated in vitro and grown on collagen sponges. 11 patients with 12 chronic deep wounds lacking dermal tissue were chosen for this study. The etiology of the ulcers were: venous stasis (6) and rh. arthritis, scleroderma and trauma (2 each). The sizes of the ulcer varied from 3.5cm2 to 46.7cm2 with a mean of 22cm2. 4 of the 6 ulcers due to venous stasis healed completely in 2 weeks and remained healed for at least 6 months. Two ulcers healed partially, 50% size reduction was achieved. Ulcers due to trauma and rh. arthritis showed 40% size reduction. Repeated grafting did not lead to complete healing. No improvement in ulcer size was seen in patients with scleroderma. The preliminary data indicate that complete or partial healing by dermal-epidermal grafts can be obtained in chronic wounds in 2 weeks. However, wounds having sub-optimal conditions for graft survival do not heal even when grafting is repeated.

TRANSDERMAL DELIVERY OF A NOVEL 5-LOI. II. DISTRIBUTION AND IN VIVO BIOLOGICAL ACTIVITY. E. Wakshull, C. Desbonnet, J. Cheng, J. Eskra, S. McNeill, and A. Kennedy. Pfizer Central Research, Groton, Ct.

We have studied the delivery characteristics of a novel 5-lipoxygenase inhibitor (5-LOI; CP-70,490) in mouse and human skin. Experiments were designed to determine the localization of the drug within the skin following topical application, and to determine whether topical application in vivo would inhibit leukotriene (LT) synthesis in a model system of inflammation. ^3H-CP-70,490, either in a 50% EtOH/H_2O vehicle or 50% EtOH/H_2O/carbopol gel formulation, was applied to mouse and human skin in vitro, or to a human skin graft (athymic mouse recipient) in vivo, respectively. The skin samples were then subjected to sequential horizontal sectioning, and the radioactivity extracted and quantitated. The results show that ^3H-CP-70,490 reached >100uM concentrations at depths of >200um into the skin, in both the in vitro and in vivo situations. HPLC analysis of the extracted horizontal sections revealed only intact drug within the skin at all depths analyzed. 5-LOI activity was assessed in vivo using as a model system the induction of LT synthesis following topical application of arachidonic acid (AA) to the mouse ear. Mouse ears were pretreated with CP-70,490 in a carbopol gel formulation (0.1, 0.3, 1mg/ml) 4hrs prior to AA challenge. Fifteen minutes after AA challenge, the ears were removed and analyzed for LT content by HPLC-RIA. At the lowest dose tested, LTB_4 synthesis was inhibited 90%, while peptido-LT synthesis was inhibited by 76%. Taken together, these results indicate that CP-70,490 possesses a nearly ideal dermal delivery profile--extremely low systemic exposure (low flux, in situ metabolism) combined with pharmacologically active concentrations delivered to the viable epidermis.

SYSTEMATIC CHANGES IN CORNEOCYTE ELEMENTAL COMPOSITION IN THE INNERMOST HUMAN STRATUM CORNEUM. R.R. Warner and N.A. Ruebusch, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, Ohio.

Human corneocytes undergo dramatic changes in elemental composition as they advance through the innermost stratum corneum (SC). Phosphorus is all but excluded from the SC, with a precipitous drop in concentration occurring at the granular/SC interface and a much smaller decline occurring over subsequent cell layers. The cellular sodium concentration (per unit volume of tissue) is generally unaltered in transit across the inner SC. The cellular potassium (K) concentration is approximately unchanged in the first SC layer, and then drops dramatically across the subsequent layer. In contrast, the cellular chloride (Cl) concentration increases in the innermost corneocyte layer, increases further in the subsequent layer or two (as K declines), and then decreases to values comparable to those in the innermost corneocyte. The increase in Cl in the second SC layer is also observed when concentrations are expressed per unit dry weight, and thus can not be explained simply by cell water loss. The position-dependent alterations in corneocyte elemental composition may reflect sequential maturation changes occurring intracellularly during SC transit.

GROWTH FACTOR EFFECTS ON CULTURED HAIR FOLLICLES. Wendy C. Weinberg, Ulrike Lichti and Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD.

This laboratory has described techniques for the enzymatic isolation of pelage hair follicles (HF) from newborn mice and their subsequent culture in a type I collagen matrix. We have studied effects of growth factors on HF cell proliferation ([^{3}H]thymidine incorporation) and collagenolytic activity (release of [^{3}H]collagen from gel matrix). The time course of [^{3}H]thymidine incorporation into HF cells is dependent on plating density, and incorporation is stimulated by TGF-α, cholera toxin and EGF. The effects are dose-dependent for each factor. Only peripheral cells of the outer root sheath proliferate as determined by autoradiography. TGF-β inhibits constitutive cell proliferation and counteracts growth stimulation by EGF or TGF-α. HF lyse the collagen gels under certain culture conditions, presumably by elaborating a type I collagenase. EGF and TGF-α stimulate gel lysis, but cholera toxin and TGF-β do not. Other skin derived cells, such as interfollicular epidermal cells, dermal fibroblasts, or combinations thereof, do not lyse gels even when stimulated by growth factors. Combinations of EGF or TGF-α with TGF-β1 or TGF-β2 are synergistic for collagenase release. Thus factors may work in concert for certain HF responses and in opposition for others. These combined actions may play a role in different phases of hair follicle development which require cell growth or invasion into the deeper dermis.

AN IMPROVED METHOD FOR THE ISOLATION AND CULTIVATION OF HUMAN SCALP DERMAL PAPILLA CELLS. Raphael Warren, Teresa K. Wong, and Karen M. Lammers, Technology Division, Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, Ohio.

An improved method for the isolation and cultivation of human scalp dermal papilla cells is presented. A comparative analysis of different culture media including those published in the literature such as Dulbecco's MEM, Eagle's MEM, Keratinocyte Growth Medium (Clonetics 'KGM') and Petra's, show that our newly identified medium, Chang, is superior. Treating the isolated dermal papilla with Type IV collagenase prior to plating in Chang medium supplemented with calf serum resulted in accelerated explant outgrowth and superior cell growth rates following cell passage. Dermal papilla cells were passaged within two weeks of explant outgrowth. The cell division rate was reduced to 2 days from its nearest competitor, Dulbecco's MEM (cell division rate of 5 days).

SUN EXPOSURE, SWIM SUIT TYPE, AND MELANOMA: RESULTS OF A COHORT-BASED CASE-CONTROL STUDY. Martin A. Weinstock, Graham A. Colditz, Walter C. Willett, Meir J. Stampfer, Ben R. Bronstein, Martin C. Mihm, and Frank E. Speizer, Dept. of Medicine, Brown Univ. and Channing Laboratory, Harvard Univ., Providence, RI and Boston, MA.

There is strong evidence that solar radiation plays a major role in the etiology of melanoma. Holman et al. found that wearing a bikini (vs. a one piece swim suit) at ages 15 to 24 was associated with risk of melanoma on the trunk (RR = 13). However, other measures of sun exposure habits (as opposed to actual sun damage, such as sunburns or actinic keratoses) have not been consistantly linked to melanoma risk. We examined the relation of sun exposure habits to melanoma risk in a nested case-control study within the Nurses' Health Study. Responses to a postal questionnaire (with telephone interview of nonrespondents) of 130 female cases age 38 to 65 years and 300 age-matched control women were analyzed by conditional logistic regression. Each of four measures of sun sensitivity were associated with melanoma risk. After controlling for sun sensitivity and latitude of residence at ages 15 to 20, the association of bikini use at ages 15 to 20 with melanoma risk was only marginally significant (RR=1.9, 95% CI=(1.0,3.7), P=.07). There was no association with risk of trunk melanoma (RR=0.8, 95% CI=(0.2,2.6), P=.8). Several measures of cumulative and intermittent intense exposure to sunlight were also unrelated to melanoma risk. We were unable to confirm any association of sun exposure habits with melanoma, and we suggest that any effect of swim suit style on risk is of much smaller magnitude than previously suggested.

REQUIREMENTS FOR HAIR GROWTH IN A NUDE MOUSE GRAFT MODEL. Wendy C. Weinberg, Ulrike Lichti, Linda Goodman*, Steve Ledbetter*, Dave Morgan, Carmen George, and Stuart H. Yuspa, Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD, and *Hairgrowth Research Unit, The Upjohn Company, Kalamazoo, MI.

Epidermal cells and cultured dermal cell populations (DCP) grafted together onto the backs of nude mice produce hairless skin (Strickland, et al. Cancer Res. 48:165, 1988). Pelage hair follicles (HF), enzymatically isolated from newborn mice, produce haired skin when grafted in combination with freshly isolated DCP (Rogers, et al. JID 89:369, 1987). We are currently exploring the necessary cell components for hair growth in this grafting system. Dissociation of HF organoids by trypsin/EDTA prior to grafting is permissive for hair growth, suggesting that follicles can reassociate *in vivo*. Both isolated HF and interfollicular epidermal cells make haired skin when grafted with uncultured DCP, but hairless skin with cultured DCP. Therefore, cells or factors present in fresh, but not cultured, dermal cell preparations are essential for supporting hair growth. Without DCP, HF grafts contain sparse hairs. Thus, cultured DCP may also inhibit hair growth. Early passage cultured dermal papilla cells, microdissected from rat vibrissal follicles, stimulate vibrissae-like hair growth in HF grafts, suggesting these cells maintain inductive capacity in culture and do not acquire inhibitory activity. Grafting of controlled cell populations promises to be useful for identifying the cellular requirements for hair growth.

STUDIES ON THE MECHANISM OF INDUCTION OF IMMUNOLOGIC TOLERANCE BY THY-1+ DENDRITIC EPIDERMAL CELLS. Elizabeth A. Welsh and Margaret L. Kripke, Department of Immunology, University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Previously, we reported that Thy-1+ dendritic epidermal cell (Tdec) lines conjugated in vitro with fluorescein isothiocyanate (FITC) induced hapten-specific immunologic tolerance upon injection into the footpad (ifp) of normal mice. Recipients of these cells were unable to develop contact sensitivity after epicutaneous sensitization. In this study we examine the mechanism by which Tdec induce immunologic tolerance. First, we determined whether other long-term, IL-2 dependent T cell lines could induce tolerance. We found that such FITC-conjugated cell lines injected ifp were unable to induce tolerance, indicating that the induction of immunologic tolerance is uniquely associated with hapten-conjugated Tdec and is not a function of long-term cell culture or IL-2 dependence. Second, we assessed the ability of DNP-conjugated Tdec to induce suppressor T cells. Spleen cells from mice injected iv of ifp with DNP-conjugated Tdec were adoptively transferred into recipient mice. Although both routes of immunization induced tolerance, suppressor T cells could be found only in the spleens of recipients injected iv with DNP-conjugated Tdec. Third, we tested for the presence of tumor necrosis factor (TNF)-like cytotoxic activity in the supernatants of the Tdec lines AU4 and AU16. Although cytotoxicity of the L929 fibroblast line was observed, AU4 and AU16 did not produce TNF_{alpha} as measured by ELISA. Fourth, we investigated whether the Tdec lines would suppress the Con A-induced proliferation of normal lymphocytes. The addition of X-irradiated Tdec to cultures of Con A-stimulated lymphocytes, at a ratio of 1:1, inhibited proliferation. Together, these results indicate that the immunologic tolerance induced by ifp injection of hapten-conjugated Tdec is not mediated by suppressor T cells, but may involve inactivation or elimination of proliferating T lymphocytes resulting in down regulation of cutaneous immune responses.

PROTECTIVE EFFECT OF ALPHA-TOCOPHEROL IN CARRIER LIPOSOMES ON ULTRAVIOLET-LIGHT-MEDIATED EPIDERMAL CELL DAMAGE *IN VITRO*. Karla Werninghaus, Rose-Marie Handjani, and Barbara A. Gilchrest, USDA Human Nutrition Research Center on Aging, Tufts Univ., Boston, MA, and Laboratoires de la Recherche, L'Oreal, Rungis, France.

We have examined the postulated protective effect of antioxidants against UV-induced epidermal damage using an *in vitro* model system. A well-differentiated squamous cell carcinoma line SCC12F2 and second passage newborn keratinocytes were maintained in a serum-free defined medium only or with carrier liposomes (LPs) alone, LPs containing 1μg/ml alpha-tocopherol (AT), or 1μg/ml AT oil alone. Cultures were irradiated once using a dose of solar simulated light (25mJ/cm^2, metered at 295nm) determined in preliminary studies to decrease cell yields after 72 hours by approximately 50% compared to sham-irradiated controls under basal conditions. Absolute cell numbers varied moderately among experiments, and hence, within each experiment, for each treatment group the ratio of cell yields for irradiated vs. sham-irradiated cultures was determined and used to analyze the effect of treatment. For SCC12F2 cells ratios were: 0.62±0.03 for untreated cells vs. 0.60±0.05 for AT oil-supplemented cells; and 0.53±0.02 for LP-supplemented cells vs. 0.66±0.03 for AT/LP-supplemented cells (8 experiments in triplicate). Compared to their LP control, AT/LP-treated cells were significantly protected (p=0.006) by an average of 25% against the decrease in cell yields induced by UV. Newborn keratinocytes had higher overall ratios of cell yields for irradiated vs. sham-irradiated cells (0.70±0.12 to 0.90±0.13, 5 experiments in triplicate), but results were otherwise similar, with AT/LP-treated cells significantly (p=0.005) protected in comparison to LP controls. We conclude that this *in vitro* system can be used to quantify UV-induced epidermal cell damage and/or repair. Our data suggest that incorporation of alpha-tocopherol into liposome preparations intended to protect sun-exposed skin may be beneficial.

HERPES SIMPLEX VIRUS DNA IS DETECTED IN SKIN LESIONS OF ERYTHEMA MULTIFORME IN CHILDREN USING THE POLYMERASE CHAIN REACTION. WL Weston, SS Stockert, JD Jester, JC Huff, AT Lane, SL Brice, Dept of Dermatology, Univ of Colorado School of Medicine, Denver, CO.

Erythema multiforme (EM) is a self-limited, often recurrent mucocutaneous disease characterized by the development of fixed, red plaques and "target lesions". EM generally afflicts adults and is less frequently seen in children. In adults, the most common precipitating factor is infection with herpes simplex virus (HSV) and HSV DNA was recently demonstrated in skin lesions of EM in a series of adult patients. The purpose of this project was to establish an association of HSV with EM in children and to examine the skin leions in this population for the presence of HSV, using the polymerase chain reaction (PCR). Six pediatric patients with EM (mean age of onset = 12 years) were included. The diagnosis of EM was based on clinical and histologic findings. A clinical history of HSV was obtained in 3. DNA was extracted from a lesional skin biopsy from each patient and examined for HSV using the PCR with HSV specific primers. HSV was detected in 4/6 specimens. These results provide evidence for an association between HSV and EM in the pediatric population, similar to that seen in adults, and may have important therapeutic implications.

EPIDERMAL CERAMIDE HYDROLASE. Philip W. Wertz and Donald T. Downing Department of Dermatology, The University of Iowa College of Medicine, Iowa City, IA

Free sphingosines, which are potential modulators of differentiation, have recently been demonstrated in epidermis. These long chain bases are present at higher concentrations in the cornified layer, where ceramides are abundant, than in the viable cells. This suggests that ceramide hydrolysis in the stratum corneum may liberate free long-chain bases, which then diffuse into the viable epidermis. The present study demonstrates an epidermal ceramide hydrolase which could mediate this reaction. In this study, a detergent-solubilized fraction from pig epidermis was incubated with a synthetic radiolabelled ceramide. After various times, reaction products were extracted into hexane, separated by thin-layer chromatography and quantitated with a linear analyzer. Under the assay conditions used, ceramide hydrolysis was linear for at least 2 h. Experiments using different enzyme dilutions indicated an endogenous dissociable inhibitor, and the activity was inhibited by exogenously supplied fatty acid. The long-chain bases liberated by this enzyme, through their known interaction with protein kinase C, may provide a means of communication between the terminally differentiated epidermal layer and the differentiating and replicative compartment.

HTLV-1 AND CUTANEOUS T-CELL LYMPHOMA. S.J.Whittaker, N.P. Smith, R. Russell Jones & L. Luzzatto. Dept.of Mol. Genetics, Hammersmith Hospital & St. John's Hospital for Diseases of the Skin, London

Genomic DNA from 40 patients with CTCL, 5 normal individuals and 5 with ATLL was examined for the presence of DNA sequences homologous to HTLV-1 using Southern blot analysis and enzymatic in vitro amplification of a specific HTLV-1 pol gene sequence. Low stringency hybridisation with an HTLV-1 probe (λ23-3) revealed identical multiple bands in both control groups and all patients reflecting the presence of homologous retroviral sequences within genomic DNA. Hybridisation at high stringency revealed discrete bands in DNA from patients with ATLL and also from 3 with CTCL, while no hybridisation was detected in other samples including normal controls. Results in ATLL were consistent with the monoclonal integration of 1 or 2 copies of HTLV-1 provirus. However in the 3 patients with CTCL, we detected an abnormal Hind III fragment in tissue DNA from 2 and a truncated 8.0kb Eco RI fragment in DNA from the third. In vitro PCR amplification of DNA samples from 5 normal individuals and 15 with CTCL was negative. In contrast, discrete bands of expected size were amplified from DNA of all patients with ATLL and 3 patients with CTCL. Hybridisation with probe λ23-3 revealed single bands and sequencing of the PCR reaction products from 2 patients with CTCL confirmed that HTLV-1 pol gene sequences were present. These results indicate that exogenous retroviral sequences can be detected in tissue DNA from a minority of patients with CTCL, but do not establish whether patients have integration of a defective HTLV-1 provirus or a different retrovirus with a high degree of homology to HTLV-1 within the pol gene.

THE TIME COURSE OF LINOLEATE METABOLISM IN PIG EPIDERMIS IN VIVO Philip W Wertz and Donald T Downing, Department of Dermatology, The University of Iowa College of Medicine, Iowa City, Iowa

Linoleic acid is essential for normal keratinization, but its function is unknown. Linoleate is present in high concentration only in the phospholipids (PL), acylglucosylceramide (AGC) and acylceramide (AC) of viable epidermis, and only in the AC of stratum corneum. The present study provides insight into the uptake, distribution, and disposition of linoleic acid in the epidermis.

Two 4-week-old pigs were each injected intradermally with 12 μCi of [U^{14}C]linoleic acid at 2 sites, at a series of separate locations, during 5 weeks. The animals were then killed and the epidermis at each site was recovered and the lipids were extracted and aliquots were resolved by TLC. Total recovered radioactivity was determined by scintillation counting, and the distribution among lipid classes was determined by scanning the chromatograms with a linear analyzer. It was found that at the first day after labelling, most of the radioactivity was contained in the phospholipids, and a small proportion in nonpolar lipids. During the next 3 weeks, the radioactivity in phospholipids declined linearly, while that in AC and AGC reached a maximum at 1 week and then declined. As a result, almost all of the labelled linoleate was lost from the system in 3 weeks. This is in marked contrast to the time course previously determined for lipids labelled through acetate, which retained all of their label for 3 weeks.

We conclude from these studies that specific pathways exist whereby linoleate is removed from the epidermis or converted to insoluble metabolites.

STIMULATION OF MELANOMA AND LYMPHOMA DEVELOPMENT BY ULTRAVIOLET RADIATION (UVR) IN HAIRLESS MICE. Michael M. Wick, Zaheed Husain and Maduhkar Pathak, Dana-Farber Cancer Institute and Dept. of Dermatology, Harvard Medical School, Boston, MA.

Clinical and epidemiological observations implicate solar UVR as a factor in the pathogenesis of human melanoma. Using topical 7,12-dimethylbenz(a)anthracene (DMBA) as an initiator and repeated exposures to UVB (290-320nm), UVA (320-400nm) or both in hairless mice (Skh-hr2), we obtained evidence to indicate that both UVA and UVB can stimulate the induction of melanomas and lymphomas as well as produce papillomas and squamous cell carcinomas (SCC). Proliferation of melanocytes in the skin could be produced with two applications of 0.5% DMBA and multiple dermal blue nevi-like lesions (10-25/animal) could be induced within 2-4 weeks. Six to eight week old mice received the following UV treatments for 30 weeks: GrI: DMBA+UVA; GrII: DMBA+UVA+UVB; GrIII: DMBA+UVB; GrIV: DMBA; GrV: UVA; GrVI: UVA+UVB; GrVII: UVB; GrVIII: control. Results indicated UVR stimulated the development of multiple blue nevi like lesions (80-100%), papillomas (56-90%), and SCCs (20-75%) in DMBA treated animals. UVB alone, or in conjunction with UVA, gave rise to a minimal number of papillomas and SCCs but no melanoma or lymphoma. Clinically recognizable melanomas were observed in 25-30% animals in groups I, II and III only. Likewise lymphomas were observed in 44-63% of animals receiving DMBA+UVR treatments. Molecular analysis revealed a 61st codon A-T transversion of the H-ras gene in all the papillomas and SCCs studied so far. This study suggests a definite role of UVR (both UVA and UVB) as a promoter in the stimulation of melanoma and lymphoma development in hairless mice.

EXTRACORPOREAL PHOTOPHERESIS FOR TREATMENT OF LYMPHOPROLIFERATIVE MALIGNANCIES. J. Wieselthier, T. Yu, T. Rothstein, H. Koh, Departments of Dermatology, Pathology and Medicine, Boston University School of Medicine, Boston, Massachusetts.

We used extracorporeal photopheresis(EP) in 15 patients with either cutaneous T-cell lymphoma(CTCL) or B-cell chronic lymphocytic leukemia (B-CLL) to study its efficacy in lymphoproliferative diseases with a malignant circulating clone. 12 CTCL patients(10 Sezary, 2 extensive plaque) were treated 2 consecutive days q month. Of the 11 evaluable patients, 6 remissions(2CR,4PR)produced an overall response rate of 55%. Mean remission duration was 4 months(range 2-7 mos.). Laboratory parameters associated with positive responses to EP include the percentage of circulating T4+cells(73.7% in responders vs. 52.3% in non-responders, $p<0.05$) and T4/T8 ratio(12.2 in responders vs. 2.66 in non-responders, $p<0.09$). 3 patients with RAI Stage 3-4 B-CLL(mean WBC 63.7K)were treated with EP 3 consecutive days q 3 weeks. After 4-6 months, all 3 stabilized their WBC count but no decrease in WBC, adenopathy or percentage of circulating lymphocytes was seen. Flow cytometry in 1 patient demonstrated increases in T4+cells(0.1 to 7.0%),T8+cells(0.9to 4.3%),T11+cells(1.9 to 10.3%) and T cell proliferative responses to PHA and ConA(4.53 to 30.98×10^{-3}cpm and 3.65 to 26.35×10^{-3}cpm, respectively). No change was noted in either B cell markers or proliferative responses to SAC. We conclude 1)our response rate in treating CTCL roughly parallels the results of Edelson et al(NEJM,316:297,1987) but other adjunctive agents are needed to improve remission duration and CR rates. 2)Photopheresis was not an effective primary treatment in this pilot study of RAI Stage 3-4 B-CLL perhaps because of intrinsic substrate differences in T and B cell diseases and/or the lack of immunologic competence and high tumor burden in these patients.

PROCREATION AND DEMOGRAPHY OF HUMAN DEMODEX MITES. Ronald D. Wise, Max Samter Institute for Allergy and Clinical Immunology, Grant Hospital of Chicago, Chicago, IL.

As the first phase of a study to investigate the possible role of Demodex antigens in collagen-vascular disease, we developed a procedure to isolate Demodex mites in human skin. Twenty-six volunteers were subjected to externally applied pressure to their facies. The blunt edge of a four-millimeter ring curette was gently pressed around the periorificial areas of pilosebaceous apparati. This resulted in the partial extrusion of the follicular contents, which were then collected within a DeLee suction catheter connected to a vacuum apparatus.

The follicular plugs were suspended in 95% ethyl alcohol and centrifuged at 1200 g. for ten minutes. The pellet was suspended in normal saline and centrifuged; this step was repeated, and the final pellet was resuspended in normal saline. Aliquots were examined microscopically at low power; 10% KOH was added to examine any particulate matter found.

Demodex mites were recovered from the facies of 6 of the 26 subjects. The mites were obtained from subjects aged 40 or older (3 men, 3 women); none of the specimens from subjects aged 16-39 contained Demodex mites. These data indicate that the human Demodex mite is not readily isolated from facial skin, in contradiction to the implication in previous publications that the Demodex mite is ubiquitous in human skin.

ADHESION OF HUMAN KERATINOCYTES TO A SYNTHETIC PEPTIDE DERIVED FROM THE α1(IV) CHAIN OF TYPE IV COLLAGEN. Mark S. Wilke and Leo T. Furcht, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN.

Based on the intimate association between the basement membrane and the basal layer of the epidermis, the present studies were aimed at further characterizing the interaction between type IV collagen and cultured human keratinocytes. To assess this interaction, direct solid phase cell binding assays were utilized in which keratinocyte adhesion to plastic substrata coated with various extracellular matrix molecules, proteolytic fragments of type IV collagen, and chemically synthesized peptides from the α1(IV) chain of type IV collagen was quantified. Initially, keratinocyte adhesion to type IV collagen was compared to other extracellular matrix molecules. In these studies, keratinocytes were found to adhere to type IV collagen to a greater degree than substrata coated with similar concentrations of fibronectin and laminin. Subsequent experiments focused on characterizing cell binding regions within type IV collagen. Type IV collagen was subjected to proteolytic digestion with pepsin, which releases the NC1 globular domain producing triple helical fragments. Substrata coated with these fragments were found to promote keratinocyte adhesion. Based on this, a series of chemically synthesized peptides derived from the α1(IV) chain of the triple helical region of type IV collagen were studied. Amoung these, one peptide designated Hep III, which is a thirteen amino acid peptide with the sequence GEFYFDLRLKGDK, was active in directly promoting keratinocyte adhesion. Furthermore, in competition assays, this peptide in solution was shown to inhibit keratinocyte adhesion to substrata coated with Hep III or intact type IV collagen. These studies show that normal cultured human keratinocytes bind directly to type IV collagen and that at least one of the cell binding sites occurs in the triple helical domain of the molecule.

CUTANEOUS $CD1^+$ CELLS EXHIBIT A NOVEL PATTERN OF CD45 (LEUKOCYTE COMMON ANTIGEN) EPITOPE EXPRESSION. Gary S. Wood and Roger A. Warnke, Departments of Dermatology and Pathology, Case Western Reserve U., Cleveland, OH and Department of Pathology, Stanford U., Stanford, CA.

The human leukocyte common antigen (LCA) consists of a family of proteins with molecular weights ranging from 180 kd to 220 kd. Monoclonal antibodies directed against these proteins are divided into four groups based on their reactivity with epitopes believed to be common to all LCA isoforms (CD45 epitopes) or restricted only to certain LCA isoforms (CD45RA, CD45RB, and CD45RO epitopes). Individual leukocytes may express more than one LCA isoform.

Using single label immunoperoxidase and double label immunoflurorescent techniques, we studied the pattern of LCA epitope expression by human cutaneous $CD1^+$ cells. The following antibodies, each specific for a different epitope, were employed: BMAC3, T29/33, 2D1, F10-89-4, 72-5D3, YTH54.12, 2B11, (all CD45 epitopes); Leu-18, 4KB5 (both CD45RA epitopes) PD7/26 (CD45RB epitope); and UCHL1 (CD45RO epitope). Of these, only the first six were reactive with cutaneous $CD1^+$ cells. This novel lack of expression of all four CD45R epitopes and one of seven CD45 epitopes is the most restricted CD45/CD45R epitope pattern reported to date and suggests that cutaneous Langerhans cells/indeterminate cells may express a novel isoform of the leukocyte common antigen. The results also indicate that two antibodies previously regarded as panleukocyte markers (PD7/26 and 2B11) are not, in fact, reactive with all bone marrow-derived leukocyte subsets, and that 2B11 probably recognizes a CD45R rather than CD45 epitope.

PSORIASIS IMPROVES WITH INFUSION OF MICROGRAM AMOUNTS OF CYCLOSPORINE A INTO LESIONS. David I. Wilkinson, Eugene M. Farber, Ellis N. Cohen, and Daniel J. Trozak, Psoriasis Research Institute, Palo Alto, Ca and Jay E. Birnbaum, Sandoz Research Institute, E. Hanover, N.J.

Injections of mg amounts of cyclosporine A (CsA) improve psoriasis, but a similar result has not been reported for ug doses that might provide the lesional levels effective during oral therapy. In this study, continuous infusion was used to deliver ug amounts to lesions.

Using 15 patients, CsA (Sandimmun I.V., diluted 25X) was infused for 2 weeks into lesions (1.5-6.0cm) at a mean rate of 8ug/hr using Alzet 2ML2 osmotic pumps mounted extracorporeally and connected by catheters to 23g needles implanted in the lesions. Adjacent control lesions were infused with either saline or Sandimmun placebo (25X dil.). Biopsies of infusion sites were taken 2 weeks after infusion for histology; sections were scored for psoriasis on a 19 point scale. All lesions were scored clinically for scaling, thickness and erythema on a 12 point scale.

An inflammatory reaction due to surfactant often distorted clinical scores; thus, biopsies were taken 2 weeks post-infusion when this had subsided. At this point, clinical scores of CsA-treated sites were lower (mean 3.2) than of control sites (placebo 4.28, saline 4.31) but with no significance ($P>0.1$). However the mean histologic score for CsA sites (5.86) was significantly less ($P<0.05$) than controls (placebo 9.85, saline 10.12).

Infusion of microgram amounts of CsA improved psoriasis both histologically and clinically, although the former assessment was more definitive.

INCREASED ANCHORING FIBRILS IN PHOTOAGED SKIN AFTER TREATMENT WITH TRETINOIN. DT Woodley, AS Zelickson, RA Briggaman, MS Hamilton, JS Weiss, JJ Voorhees: Departments of Dermatology, Stanford University, Stanford, CA, Univ of North Carolina, Chapel Hill, NC, Univ of Minnesota, Mineapolis, MN, and Univ of Michigan, Ann Arbor, MI.

Retinoic acid derivatives are known to modulate collagen synthesis and processing in vitro. Anchoring fibrils within the cutaneous basement membrane zone (BMZ) are rich in type VII collagen. The purpose of this study was to determine if topical tretinoin could modulate the number of anchoring fibrils in photoaged skin. Two millimeter punch biopsies were obtained from the forearms of 6 photoaged subjects (mean age = 50) before and after 16 weeks of "blinded", daily application of 0.1% tretinoin cream or vehicle. Specimens were processed for electron microscopy and multiple, random, non-overlapping fields of the BMZ were photographed at 31,000X by an investigator naive to the study. 248 prints were coded and evaluated by computer-assisted image analysis, and anchoring fibrils quantitated by the criteria of Tidman and Eady (J Invest Dermatol, 1984) and expressed as anchoring fibrils per linear micron of lamida densa. In the pretreatment biopsies, the mean number of anchoring fibrils was $0.77 \pm .128$, at the vehicle site and $0.64 \pm .059$ at the tretinoin sites. After daily topical treatment with tretinoin or vehicle, the anchoring fibril counts in the vehicle site were $0.65 \pm .108$ while the counts rose to $1.34 \pm .185$ in the tretinoin-treated sites. Using the 2-tailed t-test, the two-fold increase in anchoring fibrils in the tretinoin-treated sites is statistically significant ($p = 0.03$). These data suggest that a connective tissue component (anchoring fibrils) within the BMZ of human skin can be increased by the topical application of tretinoin.

CULTURE OF HUMAN KERATINOCYTES ON NORMAL HUMAN FIBROBLASTS. Peizhen Xia and Robert E. Jordon, Dept. of Dermatology, Univ. of Texas Medical School, Houston, Texas.

Human keratinocytes have been successfully grown on feeder layers of irradiated 3T3 cells. In this study, multilayered keratinocytes grew successfully on normal human fibroblasts as a feeder layer in stead of 3T3 cells. Three strains of cultured normal human newborn foreskin fibroblasts treated with mitomycin-C were used as feeder layers, and six strains of keratinocytes from different newborn foreskins ranging from first to fourth passage were plated individually on each of the three fibroblast stains. Culture medium consisted of DMEM / HAM-F12 / Adenine (DFA). After 24 hours, small keratinocyte colonies began to form. Ten to 14 days later, the cultured keratinocyte colonies became confluent and multilayered. When keratinocytes are isolated form tissue, they may be contamminated with fibroblasts; these fibroblasts interfere with keratinocyte growth. Overgrowth of fibroblasts inhibited keratinocyte colony formation so that eliminating fibroblasts from keratinocyte culture before plating is an important procedure. The present method might be applicable to human skin grafting and research of skin diseases.

OVEREXPRESSION OF IL-6 IN PSORIASIS EPIDERMIS

Hidekazu Yamada, H. Kobayashi*, Y. Aragane, M. Harada, T. Orita and T. Tezuka

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At the last SID meeting, we suggested the presence of an autocrine growth pathway by IL-6 in the activated KC and cell lines. From this point of view, K-TL-1 was stimulated by rIL-6 to detect the IL-6 mRNA overexpression. The results clearly showed that the levels of IL-6 mRNA were highly expressed after 24-48 hrs. For the model of hypertrophic growth of KC, the expression of IL-6 mRNA in the epidermis of psoriasis vulgaris (Ps) was measured. Normal epidermis, the involved skin and non-involved skin of Ps were taken by a dermatome and mRNA was prepared by the guanidine method followed by Northern blotting done with an RNA probe. In the involved skin of Ps, 0.7 Kb band was detectable, but not in either the non involved skin or the control epidermis. This band was specific for the involved skin of Ps. This means that KC in a hypertrophic state produced IL-6 and IL-6 enhanced IL-6 mRNA expression in the paracrine or autocrine manners. To prove the real autocrine growth of KC in IL-6, anti IL-6 receptor antibody (MT18, which was provided by Dr. Kishimoto) was used to stain and to block the cell growth. Also, MT18 completely blocked the growth of KC. In FACS analysis, IL-6 receptors were detectable in 10-20% of the total KC. These data support the concept that IL-6 bind the IL-6 receptor of KC, which may lead to the growth of KC. Therefore IL-6 may play a role of the autocrine growth factor or paracrine growth factor for KC.

PMA DOWN REGULATES THE EXPRESSION OF ICAM-1 ON HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS (HDMEC). Y Xu, TJ Lawley, RA Swerlick, Emory Univ, Atlanta, GA.

Phorbol 12-myristate 13-acetate (PMA) is a potent activator of protein kinase C (PKC). Stimulation of human umbilical vein endothelial cells (HUVEC) by PMA has been shown to increase the expression of ICAM-1, but these studies have not been done on small vessel endothelial cells. We have examined the regulation of ICAM-1 on HDMEC by PMA and compared it to HUVEC. As has been previously reported, ICAM-1 was constitutively expressed by both cell types, although baseline expression on HDMEC was higher. Expression of ICAM-1 was increased on both HUVEC and HDMEC by stimulation with IL-1. PMA increased the expression of ICAM-1 on HUVEC by up to 300%. In contrast, PMA decreased the expression of ICAM-1 on HDMEC by up to 60%. Down regulation of ICAM-1 by PMA was dose dependent with maximal effect at doses of 5-10 ng/ml. Increasing doses demonstrated less effect. In time course studies down regulation was seen as early as 24 hours (52%), and ICAM-1 expression continued to decrease at 72 hours (60%). PDBu, another potent PKC activator, also down regulated the expression of ICAM-1 on HDMEC (52%), while 4-O-methyl-PMA, a weak activator of PKC, failed to decrease expression (11%). The effect of PMA was inhibited by H-7, a potent inhibitor of PKC. This data demonstrates that PKC regulation of ICAM-1 on HDMEC results in a substantial decrease in ICAM-1 expression which is opposite to that seen with HUVEC.

THE ABNORMAL TRANSFER MECHANISM OF TED-H-1 ANTIGEN IN PSORIATIC SKIN. Nazuko Yamagami, Masae Takahashi, Tadashi Tezuka, Dept. of Dermatol. Kinki University School of Medicine

We previously developed a monoclonal antibody(Ted-H-1) to the keratohyalin granules(KHG) by immunizing mice with a pI 4.7, 62 KD stratum corneum membrane protein extracted from human epidermal stratum corneum. As the antigen reacted with Ted-H-1 located in vivo on the KHG and the cell membrane region of the stratum corneum (CMSC)(J.I.D. 89:400-404,1987), this antigen may have transfered from the KHG to CMSC. In order to determine if this process is a normal occurrence or not in the psoriatic skin, we have examined the distribution of Ted-H-1 antigen and actin in the KHG produced lesional skins and the treated skins of psoriasis by an indirect immunofluorescent technique. We have examined 10 psoriatic patients and 10 normal subjects. In both lesional and treated skins of all psoriatic patients, the Ted-H-1 antigen was found on the KHG but not in the cell membrane region of the stratum corneum, in which the antigen remained still granular in the cytoplasm. In contrast, the Ted-H-1 antigen located on the KHG and the cell membrane region of the stratum corneum in all normal subjects. Dotted fluorescence of the actin fibers was found on the cell membrane region in the stratum granulosum in normal skin, but this fluorescence was remarkably decreased in both psoriatic skins. Therefore, the transformation of a Ted-H-1 antigen was greatly disturbed in psoriatic skin and as a result of this, the biological function of the Ted-H-1 antigen could have been lost in the psoriatic stratum corneum.

EVIDENCE FOR NERVE GROWTH FACTOR-MEDIATED PARACRINE EFFECTS ON HUMAN MELANOCYTES. M. Yaar, K. Grossman, M. Eller, M. Peacocke and B. A. Gilchrest, USDA/ Human Nutrition Research Center, Tufts Univ., Boston, MA.

Human epidermal melanoyctes (M) express the NGF receptor (NGF-R) message and protein in vitro in response to a variety of stimuli. However, because some cells express only low affinity NGF-R and fail to respond to NGF treatment, we asked if the NGF-R inducible in M is functional and if so is there a source of NGF in the epidermis. M maintained in serum free medium were treated with phorbol 12-tetradecanoate 13-acetate for 3 days to induce the NGF-R. 50 ng/ml NGF was then added to cultures. Before and 0.5, 1, 2, 4, 8, 14 and 24 hours after NGF addition total cellular RNA was harvested for northern blot analysis. NGF transiently induced c-fos and NGF-R transcription and inhibited c-myc and actin transcription, establishing the functionality of this NGF-R. Since M become dendritic and are chemically attracted to an NGF source in vitro, mimicking their behavior in the presence of keratinocytes (K), we sought to demonstrate NGF expression by cultured K. K were deprived of growth factors for 3 days then stimulated with complete medium containing 5 ug/ml cyclohexamide to increase the anticipated low message level. Total cellular RNA was harvested after 3, 6 and 14 hours. Northern blot analysis using probes specific for the 5' as well as the 3' portions of the beta-NGF coding sequence revealed faint hybridization with mRNA of the expected 1.3 kb molecular size 14 hours after stimulation. To confirm this observation we reverse-transcribed mRNA from K samples and amplified 2 ug cDNA by sequence specific primers located in the human beta-NGF gene region using DNA thermal cycling. Electrophoresis of the product over 3% agarose gel revealed a band of the predicted size after 25 cycles. These results demonstrate that K express NGF message and that M respond to exogenous NGF stimulation by altered gene expression. These data suggest that NGF of K origin may modulate M function in normal skin.

LOCALIZATION OF COLLAGENASE AND TIMP mRNAs IN BASAL CELL CARCINOMAS. Ruth A. Yates, George Stricklin, and Lillian B. Nanney, Division of Dermatology, and Department of Plastic Surgery and Cell Biology. Vanderbilt and VA Medical Centers, Nashville, TN

The connective tissue destruction characteristic of basal cell carcinomas (BCC) is likely mediated by members of the matrix metalloproteinase family; immunohistochemical studies have demonstrated increased tissue staining for collagenase in tissue adjacent to tumor islands. Conversely, peritumor staining for tissue inhibitor of metalloproteinases (TIMP) is also increased. To localize mRNAs representing collagenase and TIMP in BCC, we employed in situ hybridization. Inserts representing collagenase and TIMP and control DNA were labeled with [35-S]-dCTP and -dATP by ramdom priming. BCCs were excised, immersed in liquid nitrogen and fixed in paraformaldehyde. We observed strong specific labeling for both collagenase and TIMP in dermis adjacent to tumor islands. Relatively few grains were noted over tumor islands. The intensity of collagenase hybridization uniformly exceeded that for TIMP. Although both fibroblasts and keratinocytes can synthesize collagenase and TIMP, our data indicate that the increased levels of these proteins in BCC likely arise from dermal cells rather than basal cells.

HYDRALAZINE INCREASES mRNAs FOR THE α AND ß SUBUNITS OF PROLYL-4-HYDROXYLASE IN HUMAN SKIN FIBROBLASTS WITH A CONCOMITANT DECREASE IN α1(I) COLLAGEN mRNAs. Heather N. Yeowell, Saood Murad, and Sheldon R. Pinnell, Division of Dermatology, Duke University Medical Center, Durham, North Carolina

Hydralazine increases prolyl-4-hydroxylase (PH) activity in cultured human skin fibroblasts whereas it substantially reduces total collagen synthesis (Arch. Biochem. Biophys. 241:356, 1985). To determine the mechanism of these effects, we measured mRNA levels for the α and ß subunits of PH and α1(I) collagen by hybridization analysis using oligonucleotide (for αPH, ßPH) and cDNA (for α1(I) collagen) probes. Treatment of cells with hydralazine (0-200 μM) for 72 h revealed a significant increase of ßPH mRNA over control (3.5 fold at ≥50 μM). In contrast, both 5.8 and 4.8 kb mRNAs for α1(I) collagen were dramatically decreased by 25 μM hydralazine. Over a period of 0-96 h, 50 μM hydralazine induced ß PH mRNA by 24 h (1.5 fold) with maximum induction at 72 h (3-4 fold). The mRNA for the α subunit of PH increased substantially after 24 h of hydralazine treatment. Both α1(I) collagen mRNAs were strongly suppressed under these conditions; the 5.8 kb mRNA was decreased >90% at 48 h and eliminated by 72 h, whereas the 4.8 kb mRNA was decreased more slowly from 65% at 48 h to complete suppression by 96 h. This study demonstrates that hydralazine not only induces mRNAs for the α and ß subunits of PH thereby leading to increased PH activity, but it suppresses both species of mRNA for α1(I) collagen effectively decreasing collagen biosynthesis.

ACCEPTANCE OF DERMAL ALLOGRAFTS IN IMMUNOCOMPETENT MICE. Karen M. Yokoo*, Charles B. Cuono*, Robert C. Langdon, and Joseph McGuire, Departments of Plastic Surgery* and Dermatology, Yale Univ. School of Medicine, New Haven , Connecticut.

Viable, cryopreserved dermal allografts have been used clinically in composite skin replacement in severe burn injury. While clinical results appear promising, the biologic behavior and fate of these dermal allografts has not been determined with certainty. The purpose of this study was to define the biologic behavior of viable, allogeneic dermis, without the overlying highly immunogenic epidermis, in a murine model. The following histocompatibility mismatch was studied: C3H/HeJ (H-2^k) versus Balb/c (H-2^d). Epidermis was removed with Dispase II, leaving only resident epidermal follicular cells. Cryopreserved skin was prepared according to standard protocols. Fresh and viable, cryopreserved C3H dermis was grafted in a subpannicular pocket in C3H(control, isogeneic graft) and Balb/c mice. The positive control was fresh, whole skin allograft, grafted in the subpannicular position. 4 grafts were placed in each mouse, and 1 graft was removed from each at days 5, 10, 18, and 40 for analysis. The positive control produced typical first-set rejection by day 10. Analysis of allogeneic dermal grafts (both fresh and cryopreserved), revealed minimum cellular infiltration, quantitatively similar to control isografts. Fresh, dermal allografts undergo focal rejection of epidermal follicular cells by 18 days. This was not seen in fresh, dermal isograft controls. Importantly, compared to fresh dermal grafts, cryopreserved dermal grafts, both isogeneic and allogeneic, exhibit quantitatively less survival of epidermal follicular cells at 5 days, and these grafts persist intact for as long as 40 days. We hypothesize that the cryopreservation process attenuates viability of follicular adnexal cells which have the potential to express class II MHC antigens. This loss of potential to express allo-class II may contribute to the long-term acceptance of cryopreserved dermal allografts.

THE CONTACT SENSITIVITY (CS) INDUCING EFFECTOR T CELL CLONE BELONGS TO A NEW MURINE $CD4^+$T CELL SUBSET. H.Yokozeki, I.Katayama, and K.Nishioka, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Recent studies have shown that murine $CD4^+$ helper T cell consist of two subsets that selectively utilize interleukin 2 (IL-2) or interleukin 4 (IL-4) as their autocrine growth factor and are called Th1 and Th2 cells, respectively. To determine the subset of the CS inducing T cell, antigen specific CS inducing T cell clone (TCL3-3) has been established and the growth characteristics of TCL3-3 clone to cytokines has been investigated. Trinitrophenyl (TNP)-specific T cell clone has been developed from lymph node cells of Balb/c mice immunized by a topical application of 7% picrylchloride (PCl) and generated by repeated stimulation with TNP-modified spleen cell (MMC treated). TCL3-3 cells have been stimulated with TNP-spleen cells in an antigen specific and MHC-restricted fashion. The phenotype of TCL3-3 is $Thy1^+$, $Lyt1^+$, $Lyt2^-$, $L3T4^+$. $7x10^6$ cells of TCL3-3 were injected subcutaneously into foodpad of naive syngeneic recipients, immediately challenged with 1%PCl. Antigen specific footpad swelling was observed after 24hrs. To investigate growth characteristics of TCL3-3, $5x10^5$ TCL3-3 cells were cultured with various cytokines (IL-1, IL-4, IL-1+IL-4, IL-2, IL-3) for 3 days. T cell proliferation was measured by ^{3}H-thymidine incorporation. TCL3-3 responded to both IL-2 (100U/ml) and IL-1 (1U/ml)+IL-4 (500U/ml). These data indicate that TCL3-3 which can induce CS has been activated by IL-4 in the presence of IL-1. On the contrary, Th1 cells which mediate delayed-type hypersensitivity doesn't proliferate to IL-4 in the presence of IL-1. We conclude TCL3-3 doesn't segregate into the Th1 and Th2 subset.

TNF-ALPHA RELEASED BY UVB-TREATED KERATINOCYTES IMPAIRS INDUCTION OF CONTACT HYPERSENSITIVITY. T. Yoshikawa and J. W. Streilein, Department of Microbiology/Immunology, University of Miami School of Medicine, Miami, Florida.

Acute, low dose ultraviolet B (UVB) irradiation of body wall skin and intradermal inoculation of tumor necrosis factor-alpha (TNF_a) both impair induction of contact hypersensitivity (CH) when dinitrofluorobenzene (DNFB) is applied to treated skin in C3H/HeN mice. Since UVB causes local synthesis and release of TNF_a in epidermis, we have tested the hypothesis that TNF_a mediates the effects of UVB on cutaneous immune responses. Panels of adult C3H/HeN mice were exposed to 4 daily doses (400 J/m^2) of UVB. Twenty four hours before the last exposure, one panel received $2x10^{-4}$ neutralizing units of anti-TNF_a antibodies. UVB-control mice received anti-BSA antibodies instead. Both panels, plus positive controls, received immunogenic DNFB epicutaneously. When ear challenged 6 days later, anti TNF_a recipients responded as vigorously as the positive controls, whereas anti-BSA recipients displayed impaired CH. To localize the locus of TNF_a action within skin, panels of mice received DNFB by epicutaneous application or by intradermal (ID) injection. Immediately thereafter, TNF_a (50 ug) was injected (ID) into the hapten-containing sites. Upon ear challenge 6 days later, vigorous CH was displayed by mice that received DNFB ID, whereas only feeble responses were detected in mice first painted epicutaneously, suggesting that TNF_a is unable to interfere with CH induction if antigen is delivered directly into the dermis. We conclude that UVB irradiation causes the local release of TNF_a from keratinocytes, and that the capacity of this cytokine to interfere with the induction of hapten-specific CH is mediated within the epidermal compartment itself, presumably on Langerhans cells.

FODRIN IS A PROLIFERATION AND MATURATION MARKER IN EPIDERMAL (EK) AND HAIR BULB KERATINOCYTES (HBK) IN VIVO AND IN VITRO. M. Younes,+ R. Paus,* K. Stenn,* I. Braverman,* and J.S. Morrow.+ Departments of Pathology+ and Dermatology*, Yale University, School of Medicine, New Haven, CT.

Fodrin is a cytoskeletal protein present at the cytoplasmic side of the plasma membrane of many cell types. In normal colonic crypts, epithelial cell maturation is associated with an increase in the abundance of fodrin (Younes et al, A J Pathol 1989). Using specific antibodies and a confocal imaging system, we found that in sections of both mouse and human skin, fodrin is membrane-associated in EK. Immuno-electron microscopy showed fodrin at the cytoplasmic side of the membrane, but excluded from the desmosomal and tonofilament attachment areas. Fodrin immunoreactivity was minimal in the basal layer EK, but increased with maturation to a maximum in the upper half of the stratum spinosum. Induction of anagen follicle development in telogen mouse skin (C57 Bl-6 model) showed a decreased fodrin immunoreactivity in the proliferating epithelial cells. In primary cultures of mouse EK at low [Ca++] fodrin had a perinuclear localization, at a higher [Ca++], fodrin localized to the cell membrane. The increased [Ca++] did not affect the apparent molecular weight of fodrin. Fodrin immunoreactivity increased in EK with stratification. Thus, the abundance of fodrin distinguishes proliferating EK from mature EK and proliferating HBK from resting HBK. EK and HBK may serve as an attractive model for studying fodrin and cytoskeletal dynamics in epithelial cells.

HUMAN EPIDERMAL MELANOCYTES HAVE LOWER PEROXIDASE ACTIVITY AND GREATER HYDROGEN PEROXIDE SENSITIVITY THAN KERATINOCYTES OR FIBROBLASTS. Yrastorza, D.G., Yohn J.J., Terada, L.S., Leff, J.A., Repine, J.E., Norris, D.A. Departments of Dermatology and Medicine, University of Colorado School of Medicine, Denver, CO

Antioxidants are vital for cellular defense against injurious and cytotoxic effects of reactive oxygen species (ROS). Ultraviolet radiation (UVR) induces production of ROS in the skin causing chronic cellular injury and promoting photocarcinogenesis and skin aging. Our recent studies have shown that cultured human epidermal melanocytes (HM) have increased susceptibility to the cytotoxic effects of hydrogen peroxide (H_2O_2) compared to keratinocytes (HK) and fibroblasts (HF). Our hypothesis is that differences in HM susceptibility to peroxide damage may be due to relatively low peroxidase activity. The purpose of this project was to quantitate peroxidase enzyme activities in HM from caucasian and black donors and compare the peroxidase activities of HM with HK and HF. We found that HF (cultured from neonatal foreskins) had significantly higher ($p<0.001$) peroxidase activity (as measured by recording the rate of H_2O_2 decomposition by ultraviolet spectroscopy) compared to neonatal foreskin HM and HK. In addition, HK had higher ($p<0.03$) peroxidase levels than HM, but no difference ($p>0.05$) in peroxidase levels was noted between caucasian and black HM. We conclude that increased HM susceptibility to damage by H_2O_2 is due, at least in part, to inadequate enzymatic defenses against H_2O_2. Decreased peroxidase activity may contribute to enhanced HM damage induced by UVR generated ROS.

cDNA AND DEDUCED AMINO ACID SEQUENCES OF MOUSE TYPE II HAIR KERATINS. Da-Wen Yu, Irwin M. Freedberg, and Arthur P. Bertolino, Hair Disease Research Laboratory, Epithelial Biology Unit, Department of Dermatology, NYU Medical Center, New York, New York.

Our mouse hair cDNA library has already provided clones that improved our understanding of Type I hair keratins. We now report two Type II hair keratin clones, MHKB-1 and MHKB-2. These clones not only provide information about this parallel class of proteins but also permit us to examine Type I and Type II hair keratin interactions.

MHKB-1 and MHKB-2 contain cDNA inserts of 634 and 1761 bp, respectively. MHKB-1 encodes from the mid-2B region of the central helical domain to the carboxy-terminus and includes the 3'-nontranslated segment. MHKB-2 includes the entire coding region for a closely related hair keratin and flanking 3'- and 5'-untranslated segments. Deduced amino acid sequences from the two clones are closely related (90%) except for carboxy terminal ends where 7 of 8 adjacent residues are distinct. Comparison of the mouse amino acid sequence encoded by MHKB-2 (479 a.a.; m.w. 52,822; pI 6.29) with a partial sheep wool keratin sequence (352 a.a.) also reveals 90% identity.

These data support the concept, already suggested by us based on Type I hair keratin relationships, that diversity within subfamilies of hair keratins is largely due to the carboxy terminal nonhelical segments. Further insight regarding the biological interrelationships among the hair keratins should emerge once antibodies to the elucidated specific epitopes are produced. These will allow us to examine the detailed tissue distribution of individual hair keratins in normal and mutant mice.

DISRUPTION OF CYTOSKELETAL MICROTUBULES BY ULTRAVIOLET RADIATION. Glen B. Zamansky and Jih-Nan Chou, Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts.

We previously demonstrated that UV irradiation of cultured dermal fibroblasts causes the depolymerization of their microtubule networks (J. Invest. Dermatol. 89, 603-606, 1987). Using indirect immunofluorescence microscopy, we have now investigated whether damage to the dimeric tubulin precursor pool may contribute to the disruption of microtubules. Repolymerization of microtubules, following disassembly by exposure to cold temperatures, is complete by 45 to 60 minutes in control cells. In sun lamp or UVA irradiated cells, repolymerization proceeds more slowly and remains incomplete 5 hours post-irradiation, many cells having a tangled mass of microtubules in the perinuclear region and only a thin network extending to the cell periphery. By exposing cells to taxol, we have also investigated the effect of irradiation on cells treated under conditions which favor assembly of microtubules. Sun lamp or UVA irradiation of taxol pretreated cells fails to cause the disassembly of microtubules. Interestingly, UV irradiation prior to taxol treatment appears to inhibit the ability of the drug to promote microtubule polymerization and bundling. In addition, we have found that UV irradiation fails to disrupt microtubule networks prepared by detergent extraction of cells. These studies indicate that damage to dimeric tubulin or another soluble factor required for polymerization contributes to the disassembly of microtubules in UV irradiated human skin fibroblasts.

CLONING AND SEQUENCING OF THE DROSOPHILA HOMOLOGUE OF A HUMAN Ro/SS-A (Ro) AUTOANTIGEN. Eugene Zappi, D.P. McCauliffe, R.D. Sontheimer and J.D. Capra, Departments of Dermatology & Microbiology, UT Southwestern Medical Center, Dallas, Tx.

There are at least four immunologically distinct Ro antigens which are recognized by autoantibodies typically found in sera from patients with Sjogren's syndrome and systemic lupus erythematosus. We recently isolated a 1.9 kb cDNA clone from a human B-cell cDNA library which encodes an acidic 46 kD Ro protein that migrates aberrantly at 52-60 kD by SDS-PAGE. The cellular function of this protein has not yet been determined although it has recently been demonstrated to be a calcium binding protein which is 94% homologous with the murine calcium binding protein, calreticulin. Southern filter hybridization analysis of drosophila DNA with the 1.9 kb Ro cDNA revealed a homologous drosophila sequence. A drosophila genomic library was screened with the 1.9 kb cDNA and a 4 kb insert was isolated. Partial DNA sequencing of this fragment showed a nucleotide sequence that is 65% homologous to the human Ro cDNA sequence when exons are compared. These portions of the drosophila sequence encode a protein whose amino acid sequence is 82% homologous with the human Ro protein. These findings confirm that this Ro protein is highly conserved from drosophila to man and suggests that it has a basic cellular function. In situ chromosomal hybridization studies with the 4 kb drosophila chromosomal fragment are in progress to determine the chromosomal position of this gene in drosophila. Further insight into the function of this gene/protein may be gained if gene mutations have been previously mapped to this position.

SERIAL T-CELL RECEPTOR GENE REARRANGEMENT ANALYSIS IN SEZARY SYNDROME. B Zelickson, SN Thibodeau, M Peters, S Muller, L Quam, M Pittelkow, Depts of Dermatology and Mol. Genetics, Mayo Clinic, Rochester, MN

T-cell receptor gene rearrangement (TCRGR) analysis is useful to establish lineage and clonality in cutaneous T-cell lymphoma (CTCL). Treatment responses in CTCL are often difficult to assess by routine clinical and laboratory criteria, including assessment of pruritus, erythema and quantitation of Sezary cells in peripheral blood. None of these parameters of response are fully satisfactory. We investigated whether serial analysis of TCRGR is a more sensitive and accurate method to quantitate treatment response in CTCL. Serial peripheral blood specimens of nine patients with CTCL were evaluated by TCRGR analysis with quantitative densitometric scanning of Southern blots. All patients were treated with extracorporeal photopheresis (ECPP). Three patients were initially treated with prednisone and chlorambucil, cyclosporine, or leukapheresis and had decreased ratios of clone/germline density prior to ECPP. One patient had decrease in erythema and absolute Sezary cell counts. Four of nine patients had an overall decrease in the ratio of clone/germline density over 4 to 17 months (ave. 11.5 mo.) that did not correlate with changes in erythema, pruritus, or absolute Sezary cell count. None of the serial TCRGR analyses showed disappearance of the clone and no patient experienced complete disease resolution. Additional TCRGRs were not detected during sequential analyses of peripheral blood specimens. Serial TCRGR analysis may be useful in detecting complete treatment responses, monitoring therapeutic efficacy during treatment, characterizing potential clonal instability with progression of CTCL, and identifying early relapse. TCRGR analysis may be combined with other laboratory measurements to quantitate extent of partial responses.

GRANULATED INFLAMMATORY CELLS IN WOUND HEALING. Brian D. Zelickson, Ellen A. Peterson, Joseph H. Butterfield and Kristin M. Leiferman, Departments of Dermatology and Medicine, Mayo Clinic, Rochester, MN.

Cutaneous wound repair involves a complex series of molecular and cellular events. To test whether neutrophils, eosinophils and mast cells infiltrate and degranulate during wound healing, specific granule proteins were localized by indirect immunofluorescence in both first and second intention wounds induced by scalpel incision or punch biopsy. Specific granule proteins, including eosinophil major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), neutrophil elastase and mast cell tryptase were detected by indirect immunofluorescence in specimens obtained after wounding up to 28 days. Although mast cells were detected in wounds at each time point in normal or slightly increased numbers (10-20/high power field) few degranulated mast cells were observed. Neutrophil degranulation was observed in early wounds (2-5 days). Eosinophils were observed in most of the wounds but were few in number (0-10/section of tissue), and there was minimal or no evidence of extracellular MBP deposition. In contrast, extracellular EDN and ECP deposition were prominent in early wounds (2-5 days). Neither mast cell numbers nor numbers of infiltrating eosinophils correlated with types of wounds or wound healing times. These results indicate that mast cell degranulation is not prominent during wound healing, whereas neutrophil degranulation occurs early. The finding of extracellular dermal EDN and ECP deposition in the absence of MBP suggests a differential release or adherence to tissues of eosinophil granule proteins during wound healing. Overall, these results suggest that neutrophil and eosinophil degranulation play a role in wound healing.

IDENTIFICATION OF A 160 KD MOLECULE AS A COMPONENT OF THE BASEMENT MEMBRANE ZONE AND AS A MINOR BULLOUS PEMPHIGOID ANTIGEN. X-J Zhu, Y Niimi, J-C Bystryn, Dept of Dermatology, NYU School of Medicine, New York, NY.

The antigens in normal human skin defined by antibodies in 20 pts with bullous pemphigoid (BP) were studied by western immunoblot techniques. Eighteen (90%) sera reacted to a 230 kD antigen present in epidermal extracts of normal human skin. Some sera also reacted to antigens with MWs of approximately 200 and 180 kDs which were not further studied. Seven (35%) sera (5 with and 2 without antibodies to the 230 kD antigens) also reacted to a 160 kD antigen. Antibodies to the 160 kD antigen were absent in 25 control sera. The 160 kD antigen was reproducibly present in epidermal extracts obtained from 4 different individuals but not in dermal extracts of the same skin specimens or in extracts of 4 control tissues (fibroblasts, melanoma, lung carcinoma, colon carcinoma). Monospecific sera with antibodies to either the 230 kD or to the 160 kD antigen reacted solely to their respective target antigens in extract of skin containing both antigens, indicating the molecules were distinct. Western blot affinity purified antibody to the 160 kD antigen bound only to the basement membrane zone (BMZ) of normal skin and to the epidermal side of split skin with 1M NaCl. The 160 kD, but not the 230 kD, antigen broke down to a 140 kD fragment in the absence of protease inhibitors.

These results indicate that a 160 kD antigen is a normal component of the BMZ of human skin. This antigen is located on the epidermal side of 1M NaCl split skin, is distinct from the 230 kD BP antigen, and is a minor BP antigen antibodies to which are present in some patients with BP.

EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR GENE IN HUMAN MELANOMA CELLS. Dimitris C. Zouzias[1], Alka Mansukhani[2], and Claudio Basilico[2], Department of Dermatology[1], and Pathology[2], New York University School of Medicine, New York, New York.

By screening a mouse cDNA library with a DNA probe corresponding to the tyrosine kinase domain of the bek gene we isolated a cDNA whose structure corresponds to that of growth factor receptors. DNA sequence analysis showed that this mouse growth factor receptor has over 90% homology with the basic fibroblast growth factor receptor (bFGFR) isolated from chicken cells. Using this bFGFR cDNA we are studying the expression of bFGFR gene in human melanoma cells. Northern blot analysis showed that some melanoma cell lines, besides the commonly found 3.4 kb mRNA, express also a smaller 1.5 kb mRNA. This smaller mRNA species corresponds to the 3' end of the bFGFR mRNA. The structure, the biogenesis and possible significance of this truncated mRNA will be discussed.